Journal of DENTAL RESEARCH

Founded by William J. Gies in 1919

Official Publication of the International Association for Dental Research

Publication Committee: H. B. G. ROBINSON, chairman, J. FRANK HALL, PAUL E. BOYLE, REIDAR SOGNNAES, NED B. WILLIAMS, AND D. Y. BURRILL.

Editor: HAMILTON B. G. ROBINSON (Ohio State University). *Business Manager:* J. FRANK HALL (Indiana University). *Circulation Manager:* PAUL E. BOYLE (University of Pennsylvania).

Assistants to the Editor: PAUL C. KITCHIN AND J. B. BROWN.

Board of Editors: MEMBERS OF THE PUBLICATION COMMITTEE,* ALSO ONE ELECTIVE REPRESENTATIVE OF EACH SECTION OF THE ASSOCIATION:

Ann Arbor, S. RAMFJORD
Austria, F. DRIAK
Baltimore, E. C. DOBBS
Birmingham, J. F. VOLKER
Boston, R. S. MANLY
Chicago, F. J. ORLAND
Cleveland, T. J. HILL
Columbus, WM. LEFKOWITZ
Denmark, J. J. HOLST
Detroit, P. C. LOWERY
Great Britain, M. A. RUSHTON
Helsinki, ------ ------
Houston, S. S. ARNIM
Indianapolis, W. G. SHAFER
Iowa City, A. K. FISHER
Johannesburg, ------ ------
Kansas City, H. L. MYERS
Louisville, D. Y. BURRILL
Materials Group, H. D. COY
Mexico, F. R. LEYCEGUI
Minnesota, W. H. CRAWFORD
Nebraska, H. W. PETERSON
New Orleans, F. J. WOLFE
New York, G. BEVELANDER
Norway, ------ ------
Pacific Northwest, B. O. A. THOMAS
Palestine, ------ ------
Philadelphia, L. I. GROSSMAN
Pittsburgh, W. F. SWANSON
Prague, ------ ------
Richmond, H. T. KNIGHTON
Rochester, B. G. BIBBY
San Francisco, W. C. FLEMING
St. Louis, T. ROSEBURY
South. Calif., B. Z. RABINOWITCH
Tennessee, O. A. OLIVER
Toronto, G. NIKIFORUK
Washington, D. B. SCOTT
Winnipeg, H. J. MERKELEY

*The Editor serves as chairman of the board, the Business Manager as secretary.

Published for THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH
By The C. V. Mosby Company, St. Louis 3, Mo.

Volume 36
1957

DENTAL LIBRARY
RK
1
.J86
copy 2

COPYRIGHT © 1957 BY THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

CONTENTS

No. 1, FEBRUARY, 1957

EDITOR'S VIEWPOINT ---------- 1

STUDIES IN THE REATTACHMENT AND REGENERATION OF THE SUPPORTING STRUCTURES OF THE TEETH. IV. REGENERATION IN EPITHELIZED POCKETS FOLLOWING THE ORGANIZATION OF A BLOOD CLOT. *W. J. Linghorne, D.D.S., M.Sc.* (University of Toronto, Toronto, Ontario) ---------- 4

THE INFLUENCE OF DIETARY THYROID ON THE BONES AND PERIODONTIUM OF RATS ON TOTAL AND PARTIAL TRYPTOPHAN DEFICIENCIES. *L. A. Bavetta, S. Bernick, and B. Ershoff.* (School of Dentistry and Medicine, University of Southern California, Los Angeles, Calif.) ---------- 13

TOOTH CONTACT DURING CHEWING. *D. J. Anderson, Ph.D., M.Sc., B.D.S., and D. C. A. Picton, B.D.S.* (Physiological Laboratory and Dental Department, Guy's Hospital, London, England) ---------- 21

TISSUE REACTION AROUND ACRYLIC ROOT TIPS. *Jens Waerhaug.* (Institute of Dental Research, Oslo, Norway, and School of Dentistry, University of Minnesota, Minneapolis, Minn.) ---------- 27

MESIODISTAL CROWN DIAMETERS OF THE DECIDUOUS AND PERMANENT TEETH IN INDIVIDUALS. *Coenraad F. A. Moorrees, Selma Ø. Thomsen, Elli Jensen, and Peter Kai-Jen Yen.* (The Forsyth Dental Infirmary for Children, Boston, Mass.) ---------- 39

HISTOLOGIC CHANGES IN THE HAMSTER CHEEK POUCH DURING EARLY HYDROCARBON CARCINOGENESIS. *John J. Salley.* (University of Rochester School of Medicine and Dentistry, Rochester, N. Y.) ---------- 48

ACID PRODUCTION FROM DIFFERENT CARBOHYDRATE FOODS IN PLAQUE AND SALIVA. *T. G. Ludwig, B.D.S., M.S., and B. G. Bibby, B.D.S., Ph.D., D.M.D.* (Eastman Dental Dispensary, Rochester, N. Y.) ---------- 56

FURTHER OBSERVATIONS UPON THE CARIES-PRODUCING POTENTIALITIES OF VARIOUS FOODSTUFFS. *T. G. Ludwig, B.D.S., M.S., and B. G. Bibby, B.D.S., Ph.D., D.M.D.* (Eastman Dental Dispensary, Rochester, N. Y.) ---------- 61

THE EFFECT OF ALLOXAN DIABETES ON CARIES INCIDENCE IN THE ALBINO RAT. *Martin S. Nichols and James H. Shaw.* (Harvard School of Dental Medicine, Boston, Mass.) ---------- 68

INHIBITION OF ACID PRODUCTION OF SALIVARY SEDIMENT BY ETHERS, ESTERS, PHENOLS, AND ALCOHOLS. *R. S. Manly, Ph.D., and Gladys Hargreaves, M.S.* (Tufts University School of Dental Medicine, Boston, Mass.) ---------- 75

SEVERAL PROPERTIES OF SALIVA FROM HUNT-HOPPERT CARIES-RESISTANT AND CARIES-SUSCEPTIBLE RATS. *S. Rosen, M. A. Benarde, F. W. Fabian, H. R. Hunt, and C. A. Hoppert.* (Michigan State University, East Lansing, Mich.) ---------- 87

DECREASED VASCULARITY OF THE DENTAL PULP. II. THE EFFECTS OF UNILATERAL LIGATION OF MAJOR VESSELS TO THE TEETH AND OF COMBINED LIGATION-SALIVARY ADENECTOMY ON DENTAL CARIES EXPERIENCE IN THE RAT. *Harold E. Brewer, Joseph C. Muhler, and Robert B. Fischer.* (Indiana University, Bloomington, Ind.) 92

EXPERIMENTAL DENTAL CARIES. IX. RELATIONSHIP OF THYROID FUNCTION TO DENTAL CARIES EXPERIENCE IN SALIVARY-ADENECTOMIZED RATS. *Joseph C. Muhler and William G. Shafer.* (Indiana University, Department of Chemistry, and School of Dentistry, Bloomington and Indianapolis, Ind.) ---------- 95

BIOCHEMICAL ANALYSIS OF HUMAN SALIVARY MUCIN. I. THE POSSIBILITY OF CHOLESTEROL IN MUCIN. *M. Irwin and A. G. Leaver.* (University of Otago Dental School, Dunedin, New Zealand) ---------- 100

EGG YOLK REACTIONS BY ORGANISMS FROM THE ORAL CAVITY OF MAN. *Genevieve D. Roth, D.D.S., and Hugh I. Myers, Ph.D.* (University of Kansas City, Kansas City, Mo.) ---------- 104

A COMPARATIVE STUDY OF HUMAN SERUM AND SALIVARY ANTIBODY TITERS IN CASES OF BRUCELLA MELITENSIS INFECTIONS. *M. G. Wheatcroft.* (U. S. Naval Medical Research Unit No. 3, Cairo, Egypt) ---------- 112

EFFECTS OF BUFFERED SOLUTIONS OF SODIUM FLUORIDE AND STANNOUS FLUORIDE ON THE SOLUBILITY OF POWDERED ENAMEL USING REPEATED DECALCIFICATION. *Robert H. Walsh, Ph.D., William H. Nebergall, Ph.D., Joseph C. Muhler, D.D.S., Ph.D., and Harry G. Day, Sc.D.* (Indiana University, Bloomington, Ind.) ---------- 118

RELATIVE CARIES-INHIBITING VALUE OF TOPICALLY APPLIED SODIUM SILICOFLUORIDE AND SODIUM FLUORIDE. *John K. Peterson, D.D.S., M.P.H., and Wm. A. Jordan, D.D.S., M.P.H.* (Minnesota Department of Health, Minneapolis, Minn.) ---------- 124

THE INHIBITORY EFFECT OF SODIUM N-LAUROYL SARCOSINATE ON ORAL LACTOBACILLI WHEN GROWN ON VARIOUS MEDIA. *I. L. Shklair, Ph.D., K. C. Hoerman, D.D.S., M.S., and H. R. Englander, D.D.S., M.P.H.* (Dental Research Facility, U. S. Naval Training Center, Great Lakes, Ill.) ---------- 129

A CLINICAL STUDY OF BLOOD PRESSURE RESPONSES TO EPINEPHRINE-CONTAINING LOCAL ANESTHETIC SOLUTIONS. *Frank M. McCarthy, M.D., D.D.S., M.S.* (Los Angeles, Calif.) ---------- 132

HISTOCHEMICAL AND ELECTRON MICROSCOPY STUDIES OF DENTIN IN TRYPTOPHAN-DEFICIENT RATS. *Sol Bernick and L. A. Bavetta.* (University of Southern California, Los Angeles, Calif.) ---------- 142

THE RELATION OF OXYGEN PRESSURE TO ENDOGENOUS OXYGEN CONSUMPTION IN BOVINE DENTAL PULP. *Alton K. Fisher, Erwin R. Schumacher, Norman P. Robinson, Delmar D. Albers, and Gary P. Sharbondy.* (State University of Iowa, Iowa City, Iowa) ---------- 150

EFFECT OF 1,2,5,6-DIBENZANTHRACENE ON TRANSPLANTED TOOTH GERMS. *Harold S. Fleming, D.M.D., M.Sc. D.Sc.* (Yale University School of Medicine, New Haven, Conn.) ---------- 153

No. 2, APRIL, 1957

EDITOR'S VIEWPOINT ---------- 163

FURTHER STUDIES ON THE INFLUENCE OF SURFACE ACTIVE AGENTS ON DECALCIFICATION OF THE ENAMEL SURFACE. *M. Irwin, A. G. Leaver, and J. P. Walsh.* (University of Otago Dental School, Dunedin, New Zealand) ---------- 166

THE EFFECT OF DIET ON THE DEPOSITION OF GLYCOPROTEIN IN THE TEETH AND ITS RELATIONSHIP TO DENTAL CARIES IN THE SYRIAN HAMSTER. *Jerome D. Buxbaum, D.D.S., Harris Kohn, D.D.S., Leah M. Proutt, M.S., and Robert H. Oster, Ph.D.* (School of Dentistry, University of Maryland, Baltimore, Md.) ---------- 173

LACTIC ACID FORMATION BY LACTOBACILLI EXPOSED TO FLUORIDIZED ENAMEL. *Jack D. Zwemer, D.D.S., Ph.D.* (Colleges of Medicine and Dentistry, University of Illinois, Chicago, Ill.) ---------- 182

THE STATE OF FLUORIDE IN DRINKING WATER. *Isaac Feldman, Donald Morken, and Harold C. Hodge.* (School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.) ---------- 192

FLUORINE IN TEETH OF ISRAELI CHILDREN, IN RELATION TO FLUORINE IN DOMESTIC WATERS AND TO DENTAL CARIES PREVALENCE. *J. Gdalia, Ph.D., and Jacob Yardeni, D.D.S.* (The Hebrew University-Hadassah Medical School, Jerusalem, Israel) ---------- 203

THE EVANSTON DENTAL CARIES STUDY. XV. THE CARIES EXPERIENCE RATES OF TWO GROUPS OF EVANSTON CHILDREN AFTER EXPOSURE TO FLUORIDATED WATER. *I. N. Hill, D.D.S., J. R., Blayney, M.S., D.D.S., W. Wolf, M.B.A.* (Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.) ---------- 208

EXPERIMENTAL CARIOSTASIS IN RATS ON A TEA DIET OF HIGH FLUORINE CONTENT. *J. F. McClendon, Ph.D., and J. Gershon-Cohen, M.D.* (Albert Einstein Medical Center, Philadelphia, Pa.) ---------- 220

A COMPARISON OF SALIVARY PROTEASE ACTIVITY IN THE HUNT-HOPPERT CARIES-RESISTANT AND CARIES-SUSCEPTIBLE RATS. *N. P. Willett, S. Rosen, H. J. Stafseth, H. R. Hunt, and C. A. Hoppert.* (Michigan State University, East Lansing, Mich.) ---------- 223

THE CHLORIDE LEVEL OF SALIVA. II. *John T. Anders.* (Forsyth Dental Infirmary for Children, Boston, Mass.) ---------- 230

EXPERIMENTAL OBSERVATION ON MELANOIDIN FORMATION IN HUMAN CARIOUS TEETH. *Samuel Dreizen, D.D.S., Ellie Jo Gilley, B.A., Janet J. Mosny, B. S., and Tom D. Spies, M.D.* (Northwestern University Medical School, Chicago, Ill.) ---------- 233

THE EFFECTIVENESS OF A TYROTHRICIN DENTIFRICE IN THE CONTROL OF DENTAL CARIES. *F. R. Shiere.* (School of Dental Medicine, Tufts University, Boston, Mass.) ---- 237

A TECHNIC FOR THE RADIOGRAPHIC VISUALIZATION OF THE VASCULAR SYSTEM OF THE PERIODONTAL TISSUES. *Philip Schuback, D.D.S., and Henry M. Goldman, D.M.D.* (The Riesman Dental Clinic, Beth Israel Hospital, Boston, Mass.) ---------- 245

THE BEHAVIOR OF TRANSSEPTAL FIBERS IN PERIODONTAL DISEASE. *Henry M. Goldman, D.M.D.* (The Riesman Dental Clinic, Beth Israel Hospital, Boston, Mass.) ------ 249

A HISTOCHEMICAL STUDY OF PROTEIN-BOUND SULFHYDRYL AND DISULFIDE GROUPS IN NORMAL AND INFLAMED HUMAN GINGIVA. *Samuel Turesky, Jeanne Crowley, and Irving Glickman.* (Tufts University School of Dental Medicine, Boston, Mass.) -- 255

ADJUNCTIVE ASCORBIC ACID ADMINISTRATION IN ANTIBIOTIC THERAPY. *Norman Freinberg, B.S., D.D.S., and Theodore Lite, B.S., D.D.S.* (Columbia Memorial Hospital, Hudson, N. Y., New York University College of Dentistry, New York, N. Y., and Jewish Chronic Disease Hospital, Brooklyn, N. Y.) ---------- 260

THE MANDIBULAR SECOND PREMOLARS: MORPHOLOGIC VARIATION AND INHERITANCE. *Fred J. Ludwig.* (Department of Anthropology, University of Arizona, Tucson, Ariz.) ---------- 263

DENTAL AUTORADIOGRAPHY. I. CHOICE OF RADIOISOTOPE. *Julian D. Boyd, M.D.* (Veterans Administration Hospital, Coral Gables, Fla.) ---------- 274

DENTAL AUTORADIOGRAPHY. II. EFFECT OF PRINTING TECHNICS ON INTERPRETATION. *Julian D. Boyd, M.D., R. W. Sims, M.Sc., and D. E. Osgood, M.A.* (Veterans Administration Hospital, Coral Gables, Fla.) ---------- 281

COMPRESSION OF TEETH UNDER THE LOAD OF CHEWING. *H. H. Neumann, M.D., and N. A. DiSalvo, D.D.S., Ph.D.* (College of Physicians and Surgeons, Columbia University, New York, N. Y.) ---------- 286

EFFECTS OF DIAPHRAGMS ON THE INTENSITY OF RADIATION IN AIR. *E. E. Petersen, D.D.S., M.S.D.* (School of Dentistry, University of Minnesota, Minneapolis, Minn.) ---------- 291

THE USE OF ANTIBIOTICS FOR THE PREVENTION OF BACTEREMIA FOLLOWING ORAL SURGERY. *Frank Holmes Cooley, M.S., D.D.S., and Sol Haberman, Ph.D.* (College of Dentistry, Baylor University, Dallas, Texas) ---------- 294

THE RELATION OF SYSTEMIC FLUORIDE AND THYROID GLAND ACTIVITY TO THE INCIDENCE OF DENTAL CARIES IN THE RAT. *David Bixler, Ph.D., and Joseph C. Muhler, D.D.S., Ph.D.* (Indiana University, Bloomington, Ind.) ---------- 304

LABORATORY METHODS FOR SCREENING POSSIBLE INHIBITORS OF DENTAL CARIES. *W. J. King, R. D. Manahan, and K. L. Russell.* (Colgate-Palmolive Co., Jersey City, N. J.) ---------- 307

THE ACTION OF INDIVIDUAL BACTERIAL STRAINS ON HUMAN TEETH UNDER IN VITRO CONDITIONS. *Ward Pigman, Edward Gilman, Ralph Powell, and Leslie Muntz.* (University of Alabama, Dental School and Medical College, Birmingham, Ala.) -- 314

No. 3, JUNE, 1957

EDITOR'S VIEWPOINT ---------- 327

COMPARATIVE MICROFLORA OF DEVELOPING DENTAL PLAQUES IN CARIES-IMMUNE AND SUSCEPTIBLE INDIVIDUALS. *Gordon E. Green, Matthew C. Dodd, and Helen S. Inverso.* (The Ohio State University, Columbus, Ohio) ---------- 331

LIPOLYTIC AND ESTEROLYTIC ACTIVITY OF SALIVA AND SALIVARY ORGANISM. *I. R. Mahler, M.S., and H. H. Chauncey, Ph.D.* (Tufts University School of Dental Medicine, Boston, Mass.) ---------- 338

THIAMINE CL-HCL EFFECT UPON ACID PRODUCTION AND UPON DEXTRINIZING TIME OF SALIVARY AMYLASE WITH CORN STARCH SUBSTRATE. *Naomi C. Turner, John T. Anders, and Norman Becker.* (Forsyth Dental Infirmary for Children, Boston, Mass.) ---------- 343

COMPARATIVE STUDIES ON THE INFLUENCE OF ANTIBIOTIC SUBSTANCES IN DENTAL CARIES IN THE WHITE RAT AND THE COTTON RAT. *James H. Shaw and Edward A. Sweeney.* (Harvard School of Dental Medicine, Boston, Mass.) ---------- 349

ANTIGENIC ANALYSIS OF STRAINS OF HETEROFERMENTATIVE LACTOBACILLI OF HUMAN ORAL ORIGIN. *Ned B. Williams and Eva B. Franck.* (University of Pennsylvania, School of Dentistry, Philadelphia, Pa.) ---------- 361

EFFECT OF 2-ACETYLAMINO-1, 3, 4-THIADIAZOLE-5-SULFONAMIDE (DIAMOX) ON DENTAL CARIES RESISTANCE IN HAMSTERS. *David F. Mitchell and Marilyn J. Johnson.* (School of Dentistry, University of Minnesota, Minneapolis, Minn.) ---------- 372

A COMPARISON OF THE SNYDER COLORIMETRIC TEST WITH THE LACTOBACILLUS COUNT USING ROGOSA MEDIUM. *Alan H. Cohen, D.D.S.* (U. S. Naval Training Center, Bainbridge, Md.) ---------- 375

THE RELATIONSHIP BETWEEN FLUORINE AND THE ACTIVITY OF THE THYROID GLAND ON THE INCIDENCE OF DENTAL CARIES IN THE RAT. *Joseph C. Muhler, D.D.S., Ph.D., David Bixler, Ph.D., and William G. Shafer, D.D.S., M.S.* (Indiana University, Department of Chemistry, Bloomington, Ind.) ---------- 382

THE METABOLISM OF DIFFERENT FLUORIDES IN THE RAT. I. COMPARISONS BETWEEN SODIUM FLUORIDE, SODIUM SILICOFLUORIDE, AND STANNOUS FLUORIDE. *Donovan A. Weddle and Joseph C. Muhler.* (Indiana University, Department of Chemistry, Bloomington, Ind.) ---------- 386

THE EFFECT OF FLUORIDE INGESTION ON THE COMPOSITION AND SOLUBILITY OF MINERALIZED TISSUES OF THE RAT. *Harold G. McCann and Frederick A. Bullock.* (National Institute of Dental Research, National Institutes of Health, Public Health Service, Bethesda, Md.) ---------- 391

EFFECT ON DENTAL CARIES OF A DENTIFRICE CONTAINING STANNOUS FLUORIDE AND DICALCIUM PHOSPHATE. *Joseph C. Muhler, D.D.S., Ph.D.* (Indiana University, Bloomington, Ind.) ---------- 399

EFFECT OF HORMONES ON STRUCTURE AND PROTEOLYTIC ACTIVITY OF SALIVARY GLANDS. *Patricia G. Clark, William G. Shafer, and Joseph C. Muhler.* (Indiana University School of Dentistry, Indianapolis and Bloomington, Ind.) ---------- 403

SURFACE CHANGES IN ENAMEL. *B. R. Bhussry, B.D.S., M.S., Ph.D., and B. G. Bibby, D.M.D., Ph.D.* (Eastman Dental Dispensary, University of Rochester, Rochester, N. Y.) ---------- 409

HISTOLOGIC CHANGES IN THE INCISOR TEETH OF RATS SERIALLY SACRIFICED AFTER RECEIVING 1,500 R OF 200 KV. X-RAY IRRADIATION. *Louis S. Hansen and James A. English.* (Naval Dental School and Naval Medical Research Institute, Bethesda, Md.) ---------- 417

STUDIES ON THE DENTITION OF THE CYNOMOLGUS MONKEY. *Aina M. Auskaps and James H. Shaw.* (Harvard School of Dental Medicine, Boston, Mass.) ---- 432

THE EFFECT OF PRESSURE ON THE HEALING OF THE EXPOSED HUMAN DENTAL PULP. *Viktors Kalnins.* (University of California College of Dentistry, San Francisco, Calif.) ---- 437

EFFECTS OF DENTAL DRUGS AND MATERIALS ON THE RATE OF OXYGEN CONSUMPTION IN BOVINE DENTAL PULP. *Alton K. Fisher, Erwin Ralph Schumacher, Norman P. Robinson, and Gary P. Sharbondy.* (College of Dentistry, State University of Iowa, Iowa City, Iowa) ---- 447

OBSERVATIONS IN VARIATION IN X-RAY DENSITY WITH BONE LOSS IN THE HUMAN MANDIBLE. *Abraham Berliner, D.M.D.* (Department of Dentistry, Sydenham Hospital, New York, N. Y.) ---- 451

INFLUENCE OF CERTAIN CONDENSATION PROCEDURES UPON THE MERCURY CONTENT OF AMALGAM RESTORATIONS. *Robert T. Wilson, Ralph W. Phillips, and Richard D. Norman.* (Indiana University School of Dentistry, Indianapolis, Ind.) ---- 458

FURTHER STUDIES ON THE CONTOUR OF CLASS II RESTORATIONS WITH VARIOUS MATRIX TECHNICS. *Cosmo Castaldi, Ralph W. Phillips, and Robert J. Clark.* (Indiana University School of Dentistry, Indianapolis, Ind.) ---- 462

STUDIES ON SYNERESIS AND IMBIBITION IN REVERSIBLE HYDROCOLLOID. *Marjorie L. Swartz, Richard D. Norman, H. William Gilmore, and Ralph W. Phillips.* (Indiana University School of Dentistry, Indianapolis, Ind.) ---- 472

AN EVALUATION OF LUBRICANTS FOR DENTAL HANDPIECES AND CONTRA-ANGLES. *G. T. Charbeneau, D.D.S., M.S., and F. A. Peyton, D.Sc.* (The School of Dentistry, University of Michigan, Ann Arbor, Mich.) ---- 479

No. 4, AUGUST, 1957

EDITOR'S VIEWPONT ---- 493

EFFECTS ON MONKEYS OF INTRODUCTION OF HEMOLYTIC STREPTOCOCCI INTO ROOT CANALS. *Donald R. Kennedy, D.D.S., Tom R. Hamilton, M.D., and Jerome T. Syverton, M.D.* (University of Minnesota, Minneapolis, Minn.) ---- 496

PLACENTAL PERMEABILITY TO BACTERIAL TOXINS. *Holmes T. Knighton, D.D.S., Seymour J. Kreshover, D.D.S., M.D., Ph.D., and James A. Hancock, Jr., M.S.* (The Medical College of Virginia, Richmond, Va.) ---- 507

FUSIFORMS IN THE INFANT MOUTH. *Valerie Hurst, Ph.D.* (University of California Medical Center, San Francisco, Calif.) ---- 513

THE EXTRA-OSSEOUS INNERVATION OF THE GINGIVAE. *Dipya Mongkollugsana, M.S., D.M.D., and Linden F. Edwards, Ph.D.* (The Ohio State University, Columbus, Ohio) ---- 516

THE RELATION OF SALIVARY SODIUM, POTASSIUM, SOLIDS AND ASH CONCENTRATION TO DENTAL CARIES EXPERIENCE IN CHILDREN, 5 TO 6 AND 12 TO 14 YEARS OF AGE. *I. Zipkin, F. A. Bullock, and N. Mantel.* (National Institute of Dental Research, National Institutes of Health, Bethesda, Md.) ---- 525

EARLY ADMINISTRATION OF VARIOUS CARBOHYDRATES AND SUBSEQUENT DENTAL CARIES IN THE RAT. *R. R. Steinman and M. I. Haley.* (College of Medical Evangelists, School of Dentistry, Loma Linda, Calif.) ---- 533

INCIDENCE OF CARIES AT DIFFERENT LOCATIONS ON THE TEETH. *John H. Barr, D.D.S., B. Sc. Dent., Ronald R. Diodati, M.S., and Russell G. Stephens, D.D.S.* (Tufts University School of Dental Medicine, Boston, Mass.) ---- 536

DENTAL CARIES AND DENTAL CARE NEEDS IN BALTIMORE SCHOOL CHILDREN (1955). *H. Berton McCauley, D.D.S., and Todd M. Frazier, A. B.* (Baltimore City Health Department, Baltimore, Md.) ---- 546

THE METABOLISM OF NATURAL AND ARTIFICIAL FLUORIDATED WATERS. *Martin J. Wagner and Joseph C. Muhler.* (Indiana University, Bloomington, Ind.) ---------- 552

THE EFFECTS OF SALIVARIADENECTOMY ON THE REPRODUCTIVE ORGANS OF THE FEMALE RAT. *David Bixler, Ph.D., Richard C. Webster, Ph.D., and Joseph C. Muhler, D.D.S., Ph.D.* (Indiana University, Bloomington, Ind.) ---------- 559

THE EFFECT OF TESTOSTERONE, THYROXINE, AND CORTISONE ON THE SALIVARY GLANDS OF THE HYPOPHYSECTOMIZED RAT. *David Bixler, Ph.D., Richard C. Webster, Ph.D., and Joseph C. Muhler, D.D.S., Ph.D.* (Indiana University, Bloomington, Ind.) ---- 566

THE RELATIONSHIP BETWEEN THE HISTOLOGY OF THE THYROID AND THE SALIVARY GLANDS AND THE INCIDENCE OF DENTAL CARIES IN THE RAT. *David Bixler, Ph.D., Joseph C. Muhler, D.D.S., Ph.D., and William G. Shafer, D.D.S., M.S.* (Indiana University School of Dentistry, Bloomington and Indianapolis, Ind.) ---------- 571

ENLARGEMENT OF SUBMANDIBULAR SALIVARY GLANDS OF RATS RESTRICTED TO ACIDIC AND BASIC SOLUTIONS AS THE SOLE SOURCE OF DRINKING FLUID. *Gerald J. Cox, Willard G. Clements, Edward C. Hilger, Jr., and David W. DeGrange.* (School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.) ---------- 576

OXIDATED ENZYMES. I. SUCCINIC DEHYDROGENASE, DPN CYTOCHROME C REDUCTASE, CYTOCHROME OXIDASE, AND CATALASE IN ORAL, LIVER, AND BRAIN CORTEX TISSUES. *Bertram Eichel and Arnold A. Swanson. With the Technical Assistance of Airman Third Class Tommie Bradley.* (Departments of Experimental Dentistry and Pathology, USAF School of Aviation Medicine, Randolph Air Force Base, Texas) ---- 581

THE MINERALIZATION OF RAT ENAMEL IN THE LIGHT OF Ca^{45} AUTORADIOGRAPHY AND MICROINCINERATION. *Leonard F. Bélanger.* (School of Medicine, University of Ottawa, Ottawa, Canada) ---------- 595

TISSUE RESPONSE TO AIRBRASIVE POWDER, TO PULVERIZED ENAMEL AND DENTIN. *Donald A. Kerr, D.D.S., M.S., Sigurd Ramfjord, L.D.S., Ph.D., and Greta Grape-Ramfjord, L.D.S., M.S.* (University of Michigan School of Dentistry, Ann Arbor, Mich) ---- 602

LOCAL EFFECT OF VARIOUS DRUGS ON THE DURATION OF INFILTRATION ANESTHESIA PRODUCED BY DENTAL ANESTHETIC SOLUTIONS. *F. P. Luduena.* (Sterling-Winthrop Research Institute, Rensselaer, N. Y.) ---------- 613

OBSERVATIONS FROM SHADOWED COLLODION REPLICAS OF TEETH WITH AMALGAM RESTORATIONS. *G. T. Charbeneau, D.D.S., M.S., and F. A. Peyton, D.Sc.* (University of Michigan, School of Dentistry, Ann Arbor, Mich.) ---------- 623

ELECTROLYTIC ACTION IN THE MOUTH AND ITS RELATIONSHIP TO PAIN. *J. M. Mumford, M.Sc., F.D.S.R.C.S.* (University of Liverpool, Liverpool, England) ---------- 632

A NEW PRINCIPLE AND APPLIANCE FOR RADIOGRAPHIC TOOTH MEASUREMENTS. *Harry H. Benkow.* (Norwegian State Dental School, Oslo, Norway) ---------- 641

DIFFERENTIAL GROWTH ANALYSIS OF VERTEBRATE TEETH. *Melvin L. Moss, D.D.S., Ph.D., and Edmund Applebaum, D.D.S.* (College of Physicians and Surgeons, Columbia University, N. Y.) ---------- 644

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH. SCIENTIFIC PROCEEDINGS OF THE JAPANESE DIVISION. *Compiled by Kei Enoki* ---------- 652

No. 5, October, 1957

EDITOR'S VIEWPOINT ---------- 655

RESOLUTION OF TRIBUTE TO DR. GIES ---------- 658

THE PRODUCTION OF DEVELOPMENTAL ABNORMALITIES OF THE ORAL STRUCTURES IN AMBLYSTOMA PUNCTATUM. *Barnet M. Levy, S. R. Detwiler, and W. M. Copenhaver.* (Columbia University Faculty of Medicine, New York, N. Y.) ---------- 659

THE STRUCTURE OF ROSTRAL TEETH AND THE ROSTRUM OF PRISTIS MICRODON. *Eric W. Bradford, D.D.Sc., M.D.S., L.D.S.* (University of St. Andrews Dental School, Queen's College, Dundee, Scotland) ---------- 663

INVESTIGATIONS OF THE INFLUENCE OF VASCULARITY AND INNERVATION ON TOOTH RESORPTION AND ERUPTION. *Barry G. Miller.* (Medical College of Virginia, School of Dentistry, Richmond, Va.) ---------- 669

THE INFLUENCE OF SYSTEMICALLY ADMINISTERED TRYPAN BLUE ON PRENATAL DEVELOPMENT OF RATS AND MICE. *Seymour J. Kreshover, Holmes T. Knighton, and James A. Hancock, Jr.* (The Medical College of Virginia, School of Dentistry, Richmond, Va.) ---------- 677

STUDIES OF THE COMPOSITION OF TEETH. VI. THE COMPOSITION OF SYRIAN HAMSTER ENAMEL AND DENTIN EXTRACTED WITH KOH-ETHYLENE GLYCOL. *George W. Burnett, D.D.S., Ph.D., and Joseph A. Zenewitz, B.S.* (Walter Reed Army Institute of Research, Washington, D. C.) ---------- 684

CLINICAL AND BACTERIOLOGIC STUDIES OF CARIES-IMMUNE HUMAN BEINGS. *Paul E. Weisenstein and Gordon E. Green.* (College of Dentistry, The Ohio State University, Columbus, Ohio) ---------- 690

THE RELATION OF STREPTOCOCCI, LACTOBACILLI, AND THE GENERAL ORAL AND FECAL FLORA TO THE PROGRESSION OF DENTAL CARIES IN THE HAMSTER. *Morrison Rogosa, Erling Johansen, and Murray N. Disraely. With the Assistance of Alfred J. Beaman.* (National Institute of Dental Research, U. S. Department of Health, Education, and Welfare, Bethesda, Md., and University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.) ---------- 695

THE EFFECT OF SEX HORMONES ON DENTAL CARIES IN DESALIVATED RATS. *David Bixler, Joseph C. Muhler, and William G. Shafer.* (Indiana University, Bloomington and Indianapolis, Ind.) ---------- 709

ENZYMES OF HUMAN SALIVA. II. PAROTID SALIVA TOTAL ESTERASES. *Howard H. Chauncey, Fabian Lionetti, and Vincent F. Lisanti.* (Tufts University School of Dental Medicine and Boston University School of Medicine, Boston, Mass.) ---------- 713

IS COLLAGENASE FOUND IN SALIVA FROM PERIODONTAL DISEASED MOUTHS? *Arnold A. Swanson, M.S.* (School of Aviation Medicine, USAF Randolph Air Force Base, Texas) ---------- 717

EFFECTS OF INHALATION OF AIRBRASIVE POWDER. *Donald A. Kerr, D.D.S., M.S., Sigurd Ramfjord, L.D.S., Ph.D., and Greta Grape-Ramfjord, L.D.S., M.S.* (University of Michigan, School of Dentistry, Ann Arbor, Mich.) ---------- 721

MICROHARDNESS STUDIES OF INTACT SURFACE ENAMEL. *Robert C. Caldwell, H.D.D., L.D.S., M.S., M. L. Muntz, D.M.D., R. W. Gilmore, B.A., D.M.D., and Ward Pigman, Ph.D.* (Medical College and School of Dentistry, University of Alabama, Birmingham, Ala.) ---------- 732

AN ABRASION METHOD FOR DETERMINING THE WEAR RESISTANCE OF TEETH. I. DESCRIPTION OF APPARATUS AND VARIABLES. *F. Taketa, H. S. Perdue, W. F. O'Rourke, H. W. Sievert, and P. H. Phillips.* (College of Agriculture, University of Wisconsin, Madison, Wis.) ---------- 739

SPECTROPHOTOMETRY OF SALIVA. *J. H. Geller, B.S., M.S., and G. H. Rovelstad, D.D.S., M.S.D.* (Dental Department U. S. Naval Training Center, Bainbridge, Md.) ---- 745

IRON CONTENT OF TEETH IN HEMOSIDEROSIS. *Benjamin H. Landing, M.D., M. E. Lahey, M.D., William K. Schubert, M.D., and Joan Spinanger.* (The Children's Hospital and Children's Hospital Research Foundation and the University of Cincinnati College of Medicine) ---------- 750

DISCOLORATION OF TEETH BY METALLIC IONS. *Sally Isaac and Finn Brudevold.* (Eastman Dental Dispensary, Rochester, N. Y.) ---------- 753

EFFECT OF NITRIC ACID AND CHELATION DEMINERALIZATION ON VARIOUS STAINS OF HISTOCHEMICAL NATURE. *Dorothy F. Mardfin and Verda Elizabeth James.* (University of Illinois College of Dentistry, Chicago, Ill.) ---------- 759

ENAMEL CARBONATE IN CARIES. *T. B. Coolidge and M. H. Jacobs.* (Department of Biochemistry and Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.) ---------- 765

THE PRODUCTION OF ACIDS FROM GLUCOSE BY ORAL MICROORGANISMS: ACIDS OF THE KREBS CYCLE. *Isaac Neuwirth and Paul J. Baerger.* (Institute for Dental Research, New York University College of Dentistry, New York, N. Y.) ---------- 769

REACTION OF TOOTH SURFACES WITH ONE PPM OF FLUORIDE AS SODIUM FLUORIDE. *F. Brudevold, J. W. Hein, J. F. Bonner, R. B. Nevin, B. G. Bibby, and H. C. Hodge.* (Eastman Dental Dispensary and University of Rochester, Rochester, N. Y.) ---- 771

THE EFFECT OF TOPICALLY APPLIED POTASSIUM FLUOROSTANNITE ON THE DENTAL CARIES EXPERIENCE IN CHILDREN. *Charles W. Gish, Joseph C. Muhler, and Charles L. Howell.* (Indiana State Board of Health, Indianapolis, Ind., and Indiana University, Department of Chemistry, Bloomington, Ind.) ---------- 780

A NEW APPROACH TO THE TOPICAL APPLICATION OF FLUORIDES FOR THE REDUCTION OF DENTAL CARIES IN CHILDREN. *Charles W. Gish, Charles L. Howell, and Joseph C. Muhler.* (Indiana State Board of Health, Indianapolis, Ind., and Department of Chemistry, Indiana University, Bloomington, Ind.) ---------- 784

THE EFFECT OF VANADIUM PENTOXIDE, FLUORIDES, AND TIN COMPOUNDS ON THE DENTAL CARIES EXPERIENCE IN RATS. *Joseph C. Muhler.* (Indiana University, Bloomington, Ind.) ---------- 787

THE GEOGRAPHICAL DISTRIBUTION OF DENTAL CARIES OF YOUNG SCHOOL CHILDREN IN THE NETHERLANDS (PERIOD 1950-1953). *J. B. Zwaardemaker and S. W. Tromp.* (Oegstgeest [Leiden], Netherlands) ---------- 795

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH. Executive Proceedings, March 21-23, 1957 ---------- 800

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH. Proceedings of the British Division, April 11 and 12, 1957 ---------- 805

No. 6, DECEMBER, 1957

EDITOR'S VIEWPOINT ---------- 819

THE RELATION OF SALIVARY CITRATE TO DENTAL CARIES EXPERIENCE IN CHILDREN 12 TO 14 YEARS OLD. *I. Zipkin, E. H. Fath, H. L. Wolff, and R. J. Fitzgerald.* (National Institute of Dental Research, U. S. Department of Health, Education and Welfare, Bethesda, Md.) ---------- 823

STUDIES ON SALIVARY LACTOBACILLI AND DENTAL CARIES IN CHILDREN DURING A DENTIFRICE TEST PROGRAM. *Gordon E. Green, Paul R. Weisenstein, and Dorothy Permar.* (Dental Research Laboratory, The Ohio State University, Columbus, Ohio) ------ 828

SALIVARY TRYPTOPHAN AND ITS RELATIONSHIP TO DMF. *Eleanor J. Edmonds, Roy W. Bell, Jr., and Ernest Beerstecher, Jr.* (Department of Biochemistry, University of Texas Dental Branch, Houston, Texas) ---------- 839

STUDIES ON SALIVARY PEROXIDATIC ACTIVITY. *Alan David Wolfe and Naomi C. Turner.* (Biochemistry Laboratory, Forsyth Dental Infirmary for Children, Boston, Mass). 843

ON THE DETERMINATION OF HUMAN SALIVA ALDOLASE ACTIVITY. *Kirk C. Hoerman and William E. Robinson.* (The Dental Research Facility, United States Naval Training Center, Great Lakes, Ill.) ---------- 852

ANXIETY AND DENTAL CARIES. *G. T. Phipps, D.D.S., and F. L. Marcuse, Ph.D.* (The State College of Washington, Pullman, Wash.) ---------- 862

SERIAL HISTOLOGIC EFFECTS OF RAT SUBMAXILLARY AND SUBLINGUAL SALIVARY GLAND DUCT AND BLOOD VESSEL LIGATION. *Samuel M. Standish and William G. Shafer.* (Indiana University School of Dentistry, Indianapolis, Ind.) ---------- 866

THE RELATION OF THYROID GLAND ACTIVITY TO THE INCIDENCE OF DENTAL CARIES IN THE RAT. II. A COMPARISON OF CARIES INCIDENCE UNDER PAIRED-FEEDING TECHNICS. *David Bixler, Ph.D., and Joseph C. Muhler, D.D.S., Ph.D.* (Indiana University, Bloomington, Ind.) ---------- 880

THE EFFECT OF PILOCARPIN ON DENTAL CARIES IN THE RAT. *Joseph C. Muhler, D.D.S., Ph.D., David Bixler, Ph.D., and William G. Shafer, D.D.S., M.S.* (Indiana University, Bloomington and Indianapolis, Ind.) ---------- 883

A COMPARISON BETWEEN SALIVARY GLAND EXTIRPATION AND DUCT LIGATION ON DENTAL CARIES IN RATS. *Joseph C. Muhler and William G. Shafer.* (Indiana University, Bloomington and Indianapolis, Ind.) ---------- 886

THE EFFECT OF DIFFERENT FLUORIDES ON THE SOLUBILITY OF INTACT DENTAL ENAMEL SURFACES. *Joseph C. Muhler.* (Indiana University, Department of Chemistry and School of Dentistry, Bloomington, Ind.) ---------- 889

THE EFFECT OF SELENIUM ON THE INCIDENCE OF DENTAL CARIES IN RATS. *Joseph C. Muhler, D.D.S., Ph.D., and William G. Shafer, D.D.S., M.S.* (Indiana University, Department of Chemistry and School of Dentistry, Bloomington and Indianapolis, Ind.) ---------- 895

A METHOD FOR THE DETERMINATION OF ENAMEL SOLUBILITY IN INTACT RAT MOLARS USING HIGHLY CONCENTRATED FLUORIDE SOLUTIONS. *Wolfgang Buttner, Dr. Med. Dent., and Joseph C. Muhler, D.D.S., Ph.D.* (Department of Chemistry, Indiana University, Bloomington, Ind.) ---------- 897

DIETARY CASEIN-SUCROSE RATIOS AND THEIR EFFECTS ON MINERALIZED TISSUES. *F. L. Losee, D.D.S., R. Van Reen, Ph.D., S. C. Peckham, M.S., W. C. Hess, Ph.D., N. Henderson, M.S., and L. J. Gerende, B.S.* (National Naval Medical Research Institute, Bethesda, Md., and Georgetown University Schools of Medicine and Dentistry, Washington, D. C.) ---------- 904

MICROSTRUCTURE OF THE HUMAN TOOTH. *F. L. Losee, D.D.S., M.S., W. H. Jennings, B.S., M. E. Lawson, Jr., and A. F. Forziati, Ph.D.* (Naval Medical Research Institute, Bethesda, Md., and National Bureau of Standards, Division of the Council on Dental Research of the American Dental Association, Washington, D. C.) ------ 911

A SOCIAL FACTOR ASSOCIATED WITH THE SEVERITY OF PERIODONTAL DISEASE. *A L. Russell.* (National Institute of Dental Research, U. S. Department of Health, Education and Welfare, Bethesda, Md.) ---------- 922

BIOCHEMICAL STUDIES ON ACUTE INFLAMMATION. II. THE EFFECT OF DILANTIN. *Bernard K. Forscher and Helene C. Cecil.* (National Institute of Dental Research, U. S. Department of Health, Education and Welfare, Bethesda, Md.) ---------- 927

A POSSIBLE MEANS OF INADVERTENT TRANSMISSION OF INFECTION TO DENTAL P ·NTS. *Jack H. Neff and S. Leonard Rosenthal.* (Temple University School of Dentistry, Philadelphia, Pa.) ---------- 932

VITAMIN A DEFICIENCY IN THE HAMSTER. *John J. Salley and William F. Bryson.* (Department of Pathology, School of Dentistry, Medical College of Virginia, Richmond, Va.) ---------- 935

A METHOD OF MEASURING POROSITY OF PORCELAIN TEETH. *John O. Semmelman.* (York, Pa.) ---------- 945

DENSIFICATION OF DENTAL PORCELAIN. *R. F. Vines and J. O. Semmelman.* (York, Pa.) ---------- 950

PROFILE CHARACTERISTICS OF CUT TOOTH SURFACES DEVELOPED BY ROTATING INSTRUMENTS. *Gerald T. Charbeneau, D.D.S., M.S., Floyd A. Peyton, D.Sc., and David H. Anthony, D.M.D.* (School of Dentistry, University of Michigan, Ann Arbor, Mich.) ---------- 957

THE EFFECT OF SURFACE AREA ON THE HYGROSCOPIC SETTING EXPANSION AND STRENGTH OF CASTING INVESTMENTS. *Joan A. Donnison, M.Sc., M. P. Chong, and A. B. Docking, M.Sc.* (Commonwealth Bureau of Dental Standards, Melbourne, Australia -- 967

THE RELATIONSHIP OF HYGROSCOPIC TO NORMAL SETTING EXPANSION OF CASTING INVESTMENTS. *A. E. Docking, M.Sc.* (Commonwealth Bureau of Dental Standards, Melbourne, Australia) ---------- 974

STUDIES ON THE SOLUBILITY OF CERTAIN DENTAL MATERIALS. *Richard D. Norman, Marjorie L. Swartz, and Ralph W. Phillips.* (Indiana University School of Dentistry, Indianapolis, Ind.) ---------- 977

STRENGTH AND PHASE FORMATION OF DENTAL AMALGAM. *Gunnar Ryge, D.D.S., R. F. Telford, D.D.S., and C. W. Fairhurst.* (Marquette University School of Dentistry, Milwaukee, Wis.) ---------- 986

RELATIONSHIP BETWEEN THE SEQUENCE OF CALCIFICATION AND THE SEQUENCE OF ERUPTION OF THE MANDIBULAR MOLAR AND PREMOLAR TEETH. *Stanley M. Garn, A.B., Ph.D., and Arthur B. Lewis, D.D.S., M.S.* (The Fels Research Institute, Antioch College, Yellow Springs, Ohio) ---------- 992

THE SHAPE OF THE DENTAL ARCHES. *James H. Scott.* (Anatomy Department, Queen's University, Belfast, Ireland) ---------- 996

INDEX ---------- 1005

36 No. 1

UNIVERSITY OF MICHIGAN
MAR 1 1957
DENTISTRY LIBRARY

Editor
HAMILTON B. G. ROBINSON

Published for
THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

By
THE C. V. MOSBY COMPANY
ST. LOUIS 3, MO.

Copyright © 1957 by The International Association for Dental Research

Journal of DENTAL RESEARCH

Founded by William J. Gies in 1919

OFFICIAL PUBLICATION OF
THE INTERNATIONAL ASSOCIATION
FOR DENTAL RESEARCH

FEBRUARY, 1957

Editor's Viewpoint ---------- 1

Studies in the Reattachment and Regeneration of the Supporting Structures of the Teeth. IV. Regeneration in Epithelized Pockets Following the Organization of a Blood Clot. W. J. Linghorne, D.D.S., M.Sc., Toronto, Ontario ---------- 4

The Influence of Dietary Thyroid on the Bones and Periodontium of Rats on Total and Partial Tryptophan Deficiencies. L. A. Bavetta, S. Bernick, and B. Ershoff, Los Angeles, Calif. ---------- 13

Tooth Contact During Chewing. D. J. Anderson, Ph.D., M.Sc., B.D.S., and D. C. A. Picton, B.D.S., London, England ---------- 21

Tissue Reaction Around Acrylic Root Tips. Jens Waerhaug, Minneapolis, Minn. ---------- 27

Mesiodistal Crown Diameters of the Deciduous and Permanent Teeth in Individuals. Coenraad F. A. Moorrees, Selma Ø. Thomsen, Elli Jensen, and Peter Kai-Jen Yen, Boston, Mass. ---------- 39

Histologic Changes in the Hamster Cheek Pouch During Early Hydrocarbon Carcinogenesis. John J. Salley, Rochester, N. Y. ---------- 48

(Contents continued on inside back cover)

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

EDITORS OF THE JOURNAL OF DENTAL RESEARCH

Publication Committee—J. Frank Hall, Paul E. Boyle, and Hamilton B. G. Robinson.

Editor—Hamilton B. G. Robinson (Ohio State University). *Business Manager*—J. Frank Hall (Indiana University). *Circulation Manager*—Paul E. Boyle (University of Pennsylvania).

Assistants to the Editor—Paul C. Kitchin and J. B. Brown.

Board of Editors—Editor emeritus, members of the Publication Committee, and sectional representatives named in the fifth column below.

THE SECTIONS—THEIR SECRETARIES, AND REPRESENTATIVES IN THE COUNCIL AND IN THE BOARD OF EDITORS (1953)

Division	*Section*	*Secretary*	*Councillor*	*Editor*
North America	Toronto	R. M. Grainger	G. Nikiforuk	H. A. Hunter
	Winnipeg	E. Roy Bier	H. J. Merkeley	E. Roy Bier
	Ann Arbor	K. Asgar	D. A. Kerr	J. K. Avery
	Baltimore	L. W. Gruber	E. C. Dobbs	L. W. Gruber
	Birmingham	L. Robinson	J. F. Volker	J. P. Lazansky
	Boston	N. C. Turner	R. S. Manly	R. O. Greep
	Chicago	H. R. Englander	F. J. Orland	G. B. Denton
	Cleveland	S. W. Chase	T. J. Hill	S. W. Chase
	Columbus	Steve Kolas	Wm. Lefkowitz	Dorothy Permar
	Detroit		P. C. Lowery	
	Houston	D. A. Hagerman	S. S. Arnim	H. Tebo
	Indianapolis	H. M. Swenson	W. G. Shafer	O. F. Mitchell
	Iowa City	K. E. Wessels	W. J. Simon	G. S. Easton
	Kansas City	Ben W. Warner	H. L. Myers	N. A. Moore
	Louisville	R. E. Myers	D. Y. Burrill	D. Y. Burrill
	Minnesota	Robert Sausen	W. H. Crawford	W. D. Armstrong
	Nebraska	D. A. Keys	D. A. Keys	R. L. Ireland
	New Orleans	H. W. Peterson	H. W. Peterson	F. J. Wolfe
	New York	P. L. Johnson	G. Bevelander	F. Krasnow
	Pacific Northwest	H. W. Fleege	B. O. A. Thomas	Ellis Jump
	Philadelphia	S. Oliet	L. I. Grossman	D. W. Cohen
	Pittsburgh	J. Nathans	W. F. Swanson	E. G. Meisel
	Richmond	J. J. Salley	H. T. Knighton	H. T. Knighton
	Rochester	F. Brudevold	B. G. Bibby	E. Johansen
	San Francisco	S. Epstein	W. C. Fleming	Hermann Becks
	St. Louis	J. R. Ring	T. Rosebury	V. H. Dietz
	Southern Calif.	L. A. Bavetta	B. Z. Rabinowitch	R. L. Rutherford
	Tennessee	J. T. Ginn	O. A. Oliver	
	Washington	W. M. Ludwick	D. B. Scott	R. Fitzgerald
	British	C. H. Tonge	M. A. Rushton	I. R. H. Kramer
Not belonging to a Division	Denmark	P. O. Pedersen	J. J. Holst	W. Krogh-Poulsen
	Helsinki	K. Koski		
	Japan	K. Enoki		
	Johannesburg	J. Staz		
	Mexico	F. R. Leycegui	F. R. Leycegui	S. Fastlicht
	Norway	G. Toverud		
	Palestine	S. Lewin-Epstein		
	Prague	F. Neumann		
	Austria, Vienna Center	E. Zitka	F. Driak	
Dental Materials Group		D. B. Mahler	H. D. Coy	N. O. Taylor

OFFICERS OF THE ASSOCIATION

President—Joseph Volker (University of Alabama), *President-Elect*—Reidar F. Sognnaes (Harvard University), *Vice-President*—Ned B. Williams (University of Pennsylvania), *Secretary-Treasurer*—E. H. Hatton (Northwestern University), *Assistant to the Secretary-Treasurer*—D. Y. Burrill (University of Louisville), *Editor*—H. B. G. Robinson, *Trustees*—H. Trendley Dean, P. C. Kitchin, T. J. Hill, H. C. Hodge, L. M. Waugh.

Vol. 36, No. 1, February, 1957. Journal of Dental Research is published bi-monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo. Subscription rates: United States and its Possessions $10.00, Students $5.00; Canada, Latin-America, and Spain $10.50, Students $5.50; Other Countries $11.00, Students $6.00. Single copies $2.50 postpaid. Entered as second-class matter June 24, 1919, at the Post Office at St. Louis, Mo., under the Act of March 3, 1879. Printed in the U. S. A.

EDITOR'S VIEWPOINT

A further study of regeneration of periodontal tissues following experimental pocket production is reported by Linghorne (p. 4). In this instance, epithelized pockets were produced in dogs and then the healing process studied. Regeneration of periodontal membrane, cementum and bone was observed. The ability of periodontium to regenerate appears to be established beyond reasonable doubt. The aim in clinical periodontics should be to find means of increasing the frequency of such regeneration; in the laboratory the mechanism should be studied in full.

Degenerative changes were examined in the long bones and periodontium of rats placed on total and partial tryptophan deficiencies and influenced by excess dietary supplements of thyroid. Bavetta, Bernick, and Ershoff (p. 13) demonstrated striking effects on bone development, including the alveolar bones, of rats. Since thyroid increases the demand for nutrient factors, in this case tryptophan, it is evident that such stress may cause the normally adequate diet to become an inadequate diet by increasing tissue demand.

Anderson and Picton (p. 21) investigated the contact of the teeth during chewing. From studies using electrical leads to an oscillograph on 10 subjects, they found that the teeth were in contact during more than half of the chewing thrusts in most individuals. If the assumption is made that occlusal disequilibrium is a factor in periodontal disease, it is important to establish whether or not contact does occur during chewing. This study establishes the fact that such contact does occur. The technic might be applied to cases in which occlusal disequilibrium is diagnosed as a factor in periodontal disease.

Waerhaug (p. 27) investigated the reaction of tissues about acrylic root tips implanted into tooth sockets. These implants were well tolerated and the proliferating epithelium which lined the socket adhered closely to the implant. Bacterial plaques on the root tips resulted in inflammation indicating a need for meticulous oral hygiene. Where natural root tips were left under the acrylic roots severe inflammation resulted.

Because of the relationship between the space occupied by the deciduous molars and the premolars, their measurements are of importance to the dentist. Moorrees, Thomsen, Jensen, and Kai-Jen Yen (p. 39) measured the mesiodistal crown diameters of these teeth on casts from 184 children. On the average, the permanent teeth exceed their deciduous predecessors in mesiodistal crown diameter, although in individual instances they may be smaller or the same size. This indicates that, usually, growth processes in the bone are necessary between the time of eruption of the deciduous molars and the time of eruption of the permanent premolars.

Salley (p. 48) reports the results of application of carcinogens to the hamster pouch. This tissue, which may be considered as accessory to the oral cavity, and equivalent, histologically, to cheek mucosa, has been established as a valuable site for experimental carcinogenesis. Salley again produced neoplasms of benign and malignant types. The disappearance of inflammatory cells from the areas of irritation after the first few treatments, in spite of continued insults, is comparable to the histologic picture of absence of cellular response (inflammatory) to chronic cheek chewing in humans. Perhaps the altered response may lead to neoplasia if, as indicated by Salley's results, continued long enough and insulted frequently enough. Salley's clear-cut results do question the often-repeated statement that experimental carcinogenesis is initiated only through hair follicles or sebaceous glands.

Ludwig and Bibby (p. 56) studied the acid production in plaque material of 6 individuals and found lower pH's resulting from eating cookies, coffee, and bread and jam than from solid foods (fried potato and apple) and from ice cream. Carbonated (cola type) beverages led to production of

little acid although 20 per cent sucrose rinse was followed by high plaque acidity. The significance of these studies is immediately suggested but one must recall that the validity of the plaque test has been questioned, the critical pH has not been established and individual habits of drinking beverages may be important. In addition, frequent consumption of sweetened beverages may be more important than relatively infrequent use of more solid foods. The subject is worthy of more investigation, especially of clinical caries in relationship to types of food.

Ludwig and Bibby (p. 61) re-evaluated some of the earlier results on foodstuff retention from the same laboratory and found variables not previously taken into consideration. Food retention was found to vary between individuals from one time to another and in the site of retention. They also propose that carbohydrate retention should be measured rather than total food retention.

Whether diabetes does or does not increase dental caries in man has been controversial. Among other factors, the rarity of completely uncontrolled diabetics has made clinical studies difficult. Nichols and Shaw (p. 68) produced experimental diabetes in rats by feeding alloxan. Many signs familiar in human diabetes appeared but caries was not significantly increased. This rat study does not solve the problem in man but gives supportive evidence to the contention that diabetes and caries may not be directly related.

Manly and Hargreaves (p. 75) used their salivary sediment method to screen 492 alcohols and phenols for acid inhibition. Monosubstituted ethers and esters were ineffective and, while alcohols and phenols generally were not active in water they were inhibitory in propylene glycol. From such a screening technic agents may be selective for more intensive and more definitive study and patently inactive substances eliminated. Even here one must use caution, for changing the solvent altered the inhibiting ability of many agents.

Rosen, Benarde, Fabian, Hunt, and Hoppert (p. 87) have investigated several properties of salivas from caries-resistant and caries-susceptible strains of rats and have found only one factor, viscosity, of even possible significance. Unfortunately, these studies have been of the nature of a survey. It is highly possible that by such methods small, but highly important differences, may be overlooked. The important development of genetically caries-resistant and caries-susceptible lines should not degenerate into an accumulation of animals but active, thorough and well-directed studies should be carried on extensively on these animals. Public funds have been used to establish this activity in a university and it is hoped that the university will recognize its responsibility to carry the work forward.

In order to determine whether or not ligation of blood vessels to the pulps of rat teeth increased caries per se, or whether the phenomenon was related to associated salivary gland effects, Brewer, Muhler, and Fischer (p. 92) ligated vessels to the pulp and also added salivary gland adenectomy in some animals. Their results indicate that interference with blood supply affects both maxillary and mandibular teeth while removal of salivary glands affects only mandibular teeth. The combined effects are additive. The mechanism of increased caries is not explained nor is the relationship between this phenomenon and the caries rate in pulpless teeth discussed.

Muhler and Shafer (p. 92) desalivated rats before giving them thyroid or thiouracil, substances which had been shown to be anticariogenic in rats with intact salivary glands. The anticaries activity of the desiccated thyroid was nullified and that of thiouracil greatly reduced. This supports the theory that the salivary glands are directly involved in the thyroid-caries relationship in the rat. The effect of thyroid activity on the salivary glands and saliva should be further defined.

Irwin and Leaver (p. 100) were unable to identify cholesterol in salivary mucin. However, salivary mucin absorbed appreciable quantities of cholesterol when shaken with it. Since differing viewpoints have been presented further study is indicated.

Roth and Myers (p. 104) found that organisms isolated from normal and inflamed mouths were capable of a lecithinase-like activity on egg yolk. Not only the well-recognized clostridia, which are infrequent oral inhabitants, but also actinomycetes and *Bacteroides melaninogenesis* were active. A Corynebacterium was positive when recombined with a streptococcus, suggesting interrelationship of the flora. The lecithinase ac-

tivity of oral organisms has not been related to any intraoral or extraoral disease.

Wheatcroft (p. 112) demonstrated positive brucella antibody titer in 80 per cent of salivas from patients with sera titers of 1:80 or over, while control salivas were negative. These studies further indicate that the antibodies were formed in the sera, not in saliva. It is suggested that saliva may be an agent for antibody loss. The absence of antibody-forming cells, such as plasma cells, in the saliva supports this viewpoint.

By decalcifying powdered enamel samples, treated with sodium fluoride and stannous fluoride solutions, three times and measuring the loss of phosphorus, Walsh, Nebergall, Muhler, and Day (p. 118) suggest that a more accurate picture of the protective abilities of the solutions is obtained. Their results indicate that these solutions are more effective at lower pH and that stannous fluoride is more effective than sodium fluoride. These studies on the pH of the solutions, however, have not yet been substituted by clinical studies.

The conclusion, from a two-year study in school children by Peterson and Jordan (p. 124), is that sodium silicofluoride is not superior to sodium fluoride when applied topically to aid in caries' prevention. On theoretical grounds, superiority had been claimed for sodium silicofluoride (Massler, *J. Dent. for Child.* 21: 14, 1954).

Sodium lauroyl sarcosinate was found to be inhibitory to lactobacilli in vitro by Shklair, Hoermann, and Englander (p. 129). The nutritive qualities of the media influenced the inhibition, indicating the importance of many factors in interpreting the possible in vivo effects of various agents on the basis of in vitro studies.

McCarthy (p. 132) studied blood pressure responses to epinephrine in local anesthetic solutions administered to human subjects. Hemodynamic pressor and heart rate responses of psychosomatic origin caused by fear were demonstrated by pseudo injections. Clinically, this suggests the value of premedication. With the palatine injection, which is somewhat painful, a mild hemodynamic pressor response was elicited, but not with other injections. Smaller amounts of epinephrine also produce hemodynamic pressor responses. These effects on normal subjects should also be tested in patients with cardiac or circulatory diseases. Such clinical studies complement and extend laboratory investigations.

Bernick and Bavetta (p. 142) studied dentin from tryptophan-deficient animals, preparing some of the material by peptic digestion before embedding and sectioning. Histochemical and electromicroscopical studies of the dentin confirmed the observation that mature dentin is highly polymerized, unlike immature dentin and dentin formed in certain deficiency states. When the ground substance is depolymerized calcification does not take place in a normal manner. The pepsin digestion seemed to produce staining qualities in dentin similar to those resulting from tryptophan deficiency.

Fisher, Schumacher, Robinson, Albers, and Sharbondy (p. 150) re-investigated the oxygen consumption of bovine dental pulp by the Warburg method. Substituting air for oxygen as the gas phase resulted in considerable variation in oxygen consumption. They suggest that this is due to "physiologic peculiarities . . . that, at present, lack adequate explanation."

Fleming (p. 153) treated transplanted tooth germs with 1:2:5:6-dibenzanthracene. Changes in the transplanted tissues included keratin formation, cyst development, and reactions of malignant character. The recent claim that the failure of transplanted tooth germs to form ameloblastomas is evidence that ameloblastomas are not of odontogenic origin is not supported by this study. It appears that odontogenic epithelium does have neoplastic potentialities. Under proper conditions, it is highly probable that the tumor formed would be an ameloblastoma.

H. B. G. R.

STUDIES IN THE REATTACHMENT AND REGENERATION OF THE SUPPORTING STRUCTURES OF THE TEETH

IV. Regeneration in Epithelized Pockets Following the Organization of a Blood Clot

W. J. LINGHORNE, D.D.S., M.Sc.

Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario

THIS is the fourth in a series of papers reporting an investigation in dogs of the controversial problem, the reattachment and regeneration of tooth-supporting structure lost through periodontal disease. The first two reports[4, 5] were basic studies under simplified experimental conditions, of the factors involved in the reattachment of the soft tissues to the tooth and the rebuilding of the bone of the socket. The present paper describes a method by which regeneration of supporting structure can be induced under experimental conditions which more closely simulate the clinical situation, i.e., in epithelized pockets resulting from the detachment and loss of supporting structure.

DISCUSSION OF THE PROBLEM

It has been known for years that regeneration of the periodontium occurs following loss of structure through injury where the gingival crevice is not involved.[1-3] The involvement of the gingival crevice introduces complicating factors that tend to produce healing at a lower level without regeneration of the lost structure. Earlier in our investigations we found that the reattachment and regeneration of surgically removed supporting structure could be induced even when the gingival crevice was involved.[4, 5] We then studied the factors in the regenerative process. However, the experimentally produced pockets differed from periodontal pockets produced by disease, in that in the latter the epithelial attachment had migrated apically and the detached gingiva was covered by epithelium on its inner surface. While much had been learned from our earlier experiments, our knowledge of the regenerative process was still inadequate to meet the more complex conditions presented by epithelized pockets. Two courses were decided upon, first, to continue work on the unknown factors in the regenerative process and, second, with epithelized pockets, to make a direct attempt to provide the conditions for inducing the desired growth of bone, connective tissue, and cementum. This paper reports the results of the second method of approach to the problem.

This investigation is supported in part by a grant to Dr. C. H. Best from The Committee on Dental Research, National Research Council, Ottawa, Canada.

Presented to the Committee on Dental Research National Research Council, Ottawa, March, 1954.

Received for publication Sept. 13, 1955.

PROCEDURE

The upper canine teeth of dogs with comparatively healthy gingival tissues were used. By means of a disc stone, a groove was cut into the tooth approximately 1 mm. from the gingival margin. This groove served as a reference point from which the level of the attachment and of the alveolar bone and the amount of recession could be measured at various stages in the experiment. A labial gingival flap was made and a section of bone and periodontal membrane, approximately 8 by 6 mm., was removed, exposing the root. The root was lightly scaled to remove any soft tissue adhering to the exposed root. A fine groove (referred to as the groove in the root to distinguish it from the gingival groove) was cut into the root outlining the bony margins of the pocket. Further loss or any regrowth of bone could then be readily ascertained either by remaking a gingival flap or by histological section. Various methods were used to prevent reattachment to the denuded root so that the oral epithelium would cover the detached gingiva and form an epithelized pocket. In the sections shown, one of two methods was used. Either the space created by removing the section of bone was filled with periodontal cement pack before the flap was sutured into place, or the denuded root was first covered with inlay cement before using the periodontal pack. The wound was allowed to heal for 45 to 90 days. In this interval, an epithelized pocket, approximately 10 by 6 mm., was formed. The measurement from the groove in the tooth to the base of the pocket was recorded. Often there was 1 to 3 mm. of recession which reduced the depth of the pocket. A gingival flap was again made sufficient to expose the denuded root and the bone at the margins. The part of the detached flap that formed the wall of the pocket appeared to be completely epithelized. Generally there was a further loss of bone, as indicated by the fine grooves placed in the root for that purpose. The usual site for the bone resorption was in the mesial or distal walls of the pocket; only occasionally was there a significant amount at the base. In 2 cases, up to 1 mm. of new bone had formed at the base. The detached gingival flap was dried as far as possible and covered with a solution of gum copal in alcohol, and a few minutes later with collodion ("New Skin"). All soft tissue attached to the tooth at the margin above the bone was removed. The bony margins of the pocket were freshened and the tooth scaled until it appeared fresh and clean. The root was then covered by autogenous bone grafts (small chips) secured from a neighboring tooth socket. Sufficient grafts were used to completely fill the space to the level of the adjacent bone. The flap was sutured into place, care being taken to make sure a blood clot had formed covering the grafts. Collodion was used to protect the blood clot. After a healing period, varying from 45 to 100 days, a gingival flap was again laid back to ascertain the amount of regeneration. If sufficient reattachment and regeneration of bone had occurred (4 to 7 mm.) the flap was resutured and left to heal for about 30 days, and blocks were then removed for sectioning. If there was little or no regeneration, the procedures outlined were repeated.

Various means were used to make sure that epithelization of the pocket was completed in the interval allowed for that purpose. Longitudinal sections of tooth and pocket wall were made and it was found that epithelization was usually complete in 35 days. Additional time, 45 to 90 days, was therefore allowed. Again, in order to test this important feature in the cases destined to be carried on to completion, the tissue attached to the tooth above the bone at the base of the epithelized pocket was removed, sectioned, and examined for the presence of epithelium. Its presence in this tissue would indicate that epithelization of the detached gingiva to the base had occurred.

RESULTS

Table I shows the amount of reattachment and regeneration of bone on 11 teeth. The figures were arrived at by the following method. By means of a probe, graduated in millimeters, the measurement was made from the gingival groove to the line of attachment at the base of the epithelized pockets. The same measurement was made after the completion of the regenerative procedures. The amount of reattachment was the difference between these figures. However, the level of the reattached gingiva was not uniform across the width of the pocket. Generally but not always it was higher at the sides and lower in the center. The figure recorded represents the average level.

TABLE I

DOG	TOOTH	AMOUNT OF NEW BONE (MM.)	AMOUNT OF REATTACHMENT (MM.)
1	4	6	6
4	4	1 - 2	5
	4	4 - 6	5
6	4	5 on side	6
7	4	4½	6
8	4	4	4½
	4	5½	7
9	4	4	5
	4	2	4
10	4	7 4	5
	4	4	4

Thus, histological sections may show more or less reattachment than was recorded in Table I. By remaking a gingival flap after the completion of the regenerative process, the amount of new bone could be similarly measured. Again the level of the new bone was not uniform across the width of the pocket. The figures in the table represent the average amount of bone regeneration. In a few cases, two measurements are given to describe more adequately the amount of bone regeneration.

Observations on the Living Animal.—Usually where regeneration had occurred the gingival crevice was 2 to 3 mm. in depth. This favorable depth was achieved by both regeneration and recession. When the gingival flap was made early in the healing period (45 days following the procedures aimed

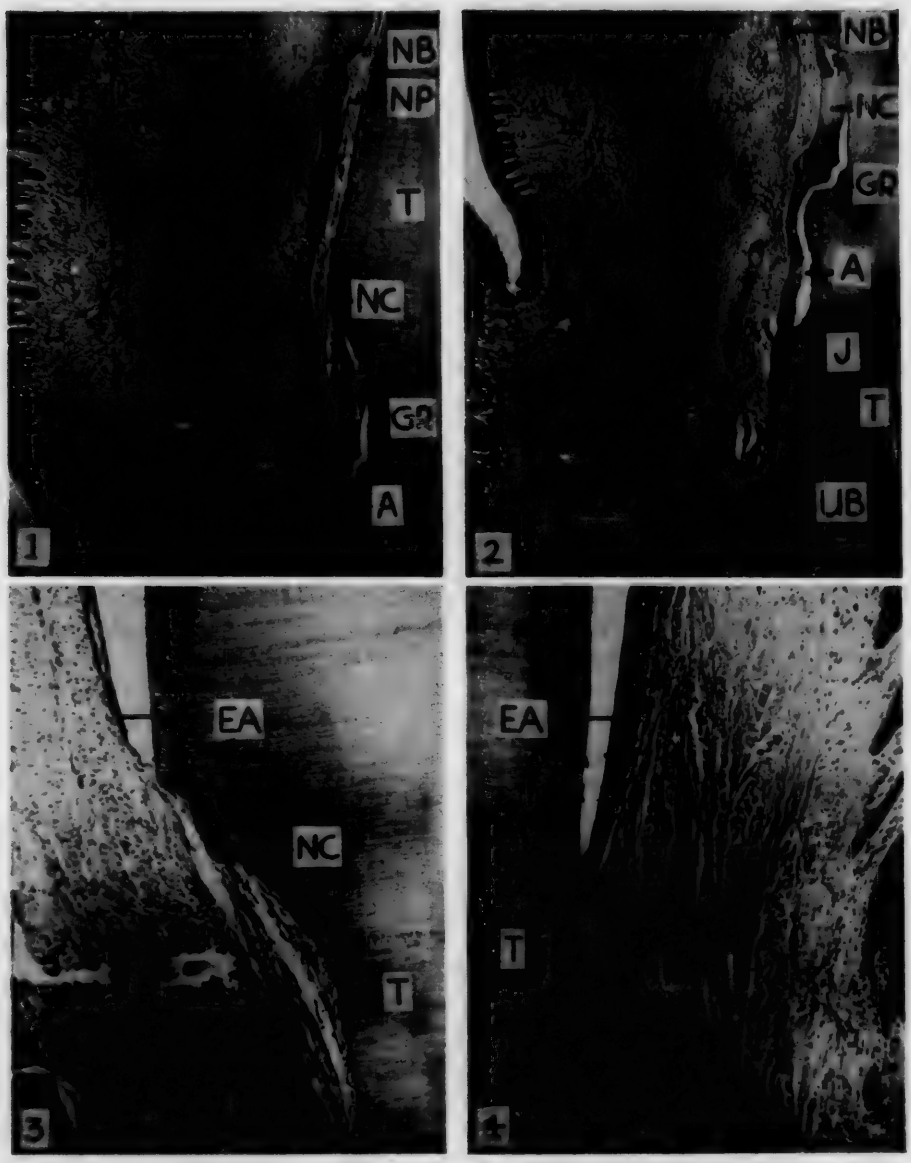
at inducing regeneration) the new bone was clearly outlined as it was darker in color than the adjacent undisturbed bone. Besides being darker in color, the young bone could be easily pierced with a blunt probe. In our most successful case, 7 mm. of new bone were induced.

Histological Observations.—In the reattachment process, resorption of both cementum and dentin on the denuded root occurred prior to the differentiation of cementoblasts and the apposition of new cementum. A comparison of Fig. 3 with Fig. 4 illustrates the changes in the outline of the root. Thus, in the histological sections a wavy irregular outline of the root surface caused by its resorption indicates the area that had been denuded. It follows then that any bone, periodontal membrane, or cementum opposite such a resorbed root surface must be new tissue; the alveolar process and periodontal membrane must obviously have been removed before the root could be denuded. In longitudinal sections supporting structure crownwise to the groove in the root must also be new tissue.

Fig. 1 is a photomicrograph of a section from Dog 7. The magnification is too low for detail but permits a field approximately 5 mm. in diameter. It shows the fine groove in the root at the base of the pocket and most of the new bone and new periodontal membrane. In this section, 7 mm. of reattachment and 5 mm. of new bone were achieved in an epithelized pocket, 11 mm. in depth by 7 mm. The alveolar process is quite thin in this animal but in Fig. 2, a field apical to Fig. 1, showing both new and undisturbed bone, it is apparent that the new bone corresponds in thickness to the original bone. Fig. 3 is a higher magnification of the gingival area of this case. The amount of resorption of the root occurring in the reattachment process is apparent when Fig. 3 is compared with Fig. 4, the corresponding undisturbed area on the lingual of the same tooth. Fig. 5 is a low-power magnification at the base of the lesion. Notice the increased thickness of the new cementum in the groove, a constant observation throughout this study.

Fig. 6 is a low-power magnification of the gingival area in Dog 8. Here the new bone is thicker than the original bone. The distance from the epithelial attachment to the alveolar crest is 1 mm., being ½ of the normal distance (2 mm. in the dog). In this case the new periodontal membrane shown in Fig. 7 appears as usual to be composed of connective tissue. However, in Fig. 8, a field apical to Fig. 7, the new periodontal membrane is more like bone marrow than periodontal membrane. The probable reason for this observation can be seen in Fig. 9 which is from a section a little deeper into the block. Here we see part of the new bone is ankylosed to the tooth, thus changing the function of the adjacent newly formed tissue of the periodontal membrane.

The case with the shortest healing time, 104 days, is shown in Fig. 10. Approximately ⅞ of the bone was regenerated. Remnants of the epithelium, lining the soft tissue wall of the pocket, are still present as islands. These



Key to abbreviations on Figs. 1-12.

NB, New bone.
UB, Undisturbed bone.
NP, New peridontal membrane.
NC, New cementum.
GR, Groove in the root.
EA, Epithelial attachment.
PE, Pocket epithelium.
NF, New connective tissue fibers.
J, Junction of old and new attachments.
T, Tooth.
E, Epithelium.
A, Artifact

Fig. 1.—Dog 7. Healing time, 113 days. Pocket 11 by 7. Reattachment 7 mm. New bone 5.5 mm. Notice the groove cut into the root to mark the base of the pocket. Orig. mag. ×50.

Fig. 2.—Dog 7. Showing field apical to that in *Fig. 1*. Notice that the new bone is comparable in thickness to the undisturbed bone in the lower part of the field. Notice the normal outline of the cementodentinal junction in the undisturbed area below *J*. Orig. mag. ×50.

Fig. 3.—Dog 7. View at gingival of reattached area. Notice the amount of resorption of old cementum and dentin that occurred in the reattachment process. Orig. mag. ×110.

Fig. 4.—Dog 7. View of gingival area on untouched lingual of same tooth. Notice the smooth outline of the cementoenamel junction when compared with the resorbed outline in *Fig. 3*. Orig. mag. ×110.

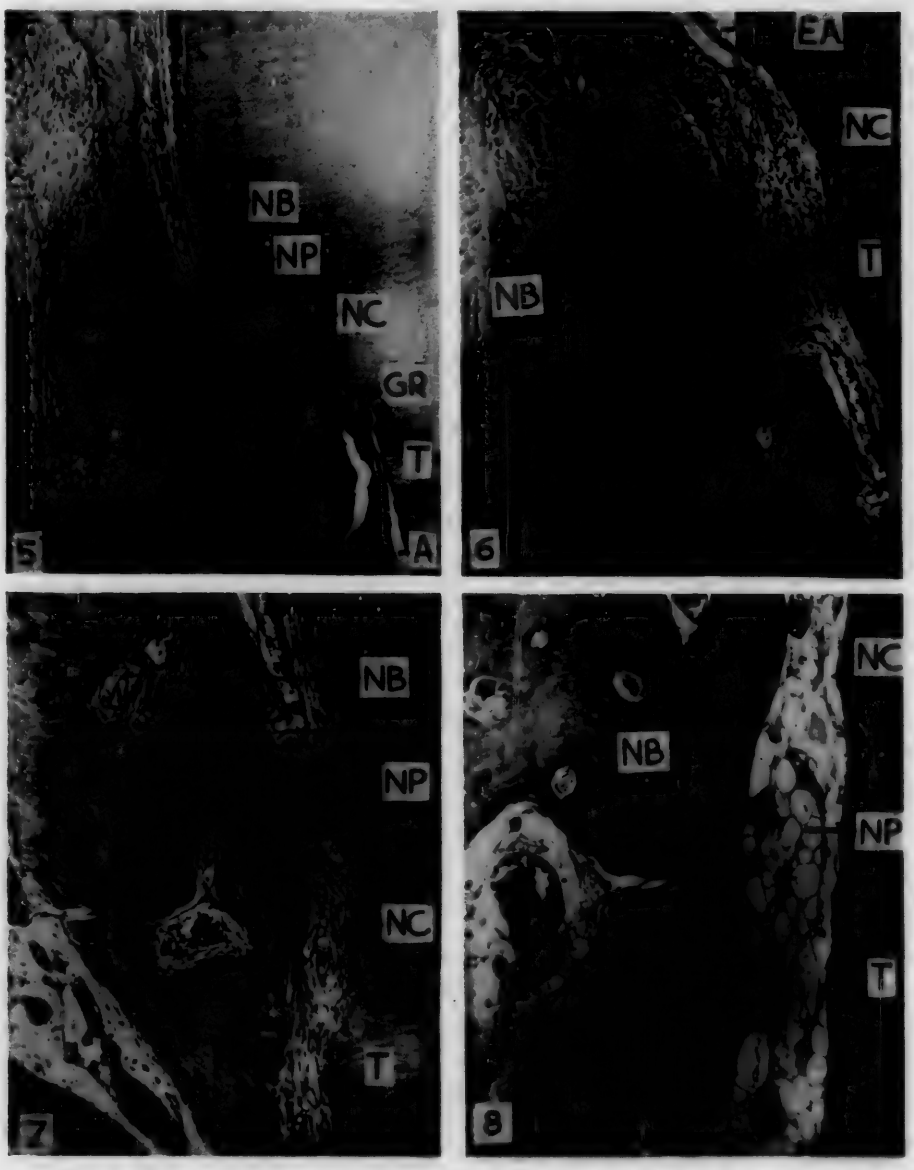
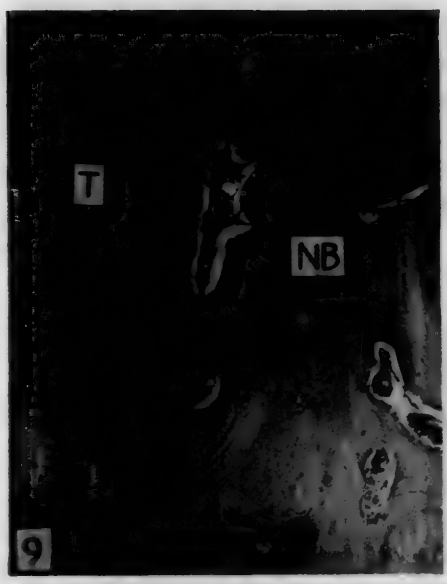


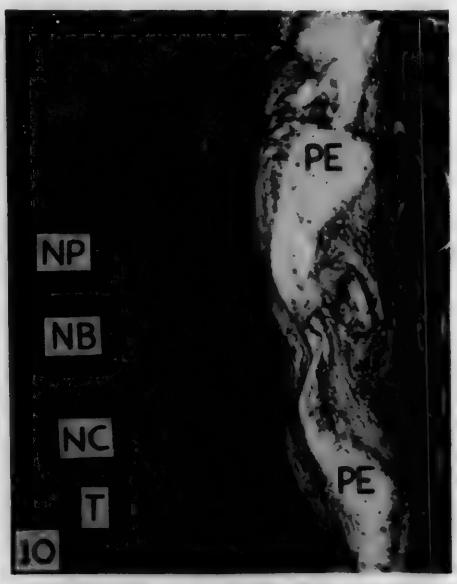
Fig. 5.—Dog 7. Higher magnification of the field shown in *Fig. 1*. Notice that cellular cementum is deposited on the resorbed root, and the increased thickness in the groove. Orig. mag. ×90.

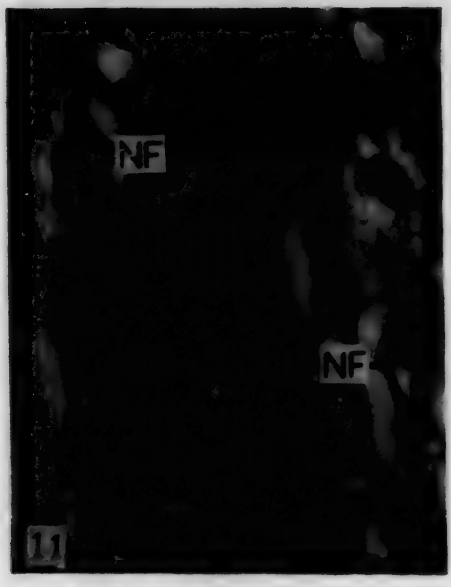
Fig. 6.—Dog 8. Healing time 117 days. At this stage the new bone is considerably thicker than the original bone. From the epithelial attachment to the new bone measured 1 mm. which is ½ the normal measurement in the dog. Orig. mag. ×90.

Fig. 7.—Dog 8. Field apical to field in *Fig. 6*. Notice the characteristic fibrous tissue in the new periodontal membrane. Orig. mag. ×90.

Fig. 8.—Dog 8. This field is from the same section but apical to that in *Fig. 7*. The new periodontal membrane is like bone marrow. Orig. mag. ×90.







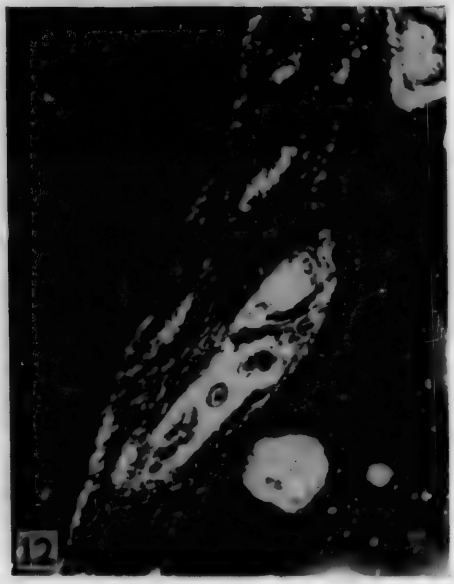


Fig. 9.—Dog 8. Showing a field from a section a little deeper into the block. Bone and tooth are ankylosed. Orig. mag. ×90.

Fig. 10.—Dog 1. Healing time, 104 days. The pocket epithelium is degenerating but as yet is not replaced by fibrous tissue. Orig. mag. ×110.

Fig. 11.—Dog 1. Higher magnification of degenerating epithelium in *Fig. 10* being invaded by new fibrous tissue. Orig. mag. ×1060, oil immersion.

Fig. 12.—Section stained with Mallory's connective tissue stain. Notice the oblique direction of the principal fibers of the newly formed periodontal membrane.

islands are in the process of being replaced by fibrous tissue, as can be seen with the higher magnification in Fig. 11. In the cases with longer healing periods, the epithelium had largely disappeared.

Fig. 12 is from a section of Dog 8 stained by Mallory's connective tissue stain. The oblique direction of the principal fibers of the new periodontal membrane indicates that orientation of the fibers to the forces of occlusion has taken place. When reattachment occurs without regeneration of bone, the connective tissue fibers run parallel to the tooth.

DISCUSSION

One of the objectives of this investigation is to ascertain whether or not supporting structure lost through periodontal disease can be regenerated. If this is found to be the case, our plan is to study the physiology of the regenerative process in order that clinical procedures may be developed on a rational basis. After studying the evidence presented in this paper, it was decided to postpone a discussion of the manner in which the regeneration took place as we felt that further work was required to throw more light on certain fundamental factors. Our purpose in this paper is to show that at least in dogs, regeneration of supporting structure may occur from epithelized pockets following the organization of a blood clot, and second, to indicate that this regeneration may occur without first removing the pocket epithelium from the soft tissue wall of the pocket. We would like to make it clear that the procedures described in this paper are not offered as a clinical method for inducing regeneration.

For many years it has been considered essential that the pocket epithelium and the epithelial attachment be removed to the last remnant, if there is to be any hope of reattachment. The technical difficulties in removing the pocket epithelium are such as to be quite discouraging to the clinician. Our studies suggest that this difficult task may not be necessary after all, as regeneration was induced without any attempt to remove the epithelium. In fact, it is quite possible that the pocket epithelium serves a useful purpose in the regenerative process.

In all of the successful cases the new cementum was thicker in the groove in the root than on the adjacent areas of the root. It is our belief that the stimulus for this increased deposition is functional, but as yet the mechanism is not well understood.

It appears now that regeneration of lost supporting structure may be induced in dogs by two methods, namely, by organization of a blood clot comparable to the healing of a fracture as described in this paper, and by a much slower method, probably dependent, to some extent at least, on an inherent growth potential in these tissues. This method was described in study.[3, 6]

CONCLUSIONS

1. In dogs, experimental reattachment and regeneration of bone and periodontal membrane was induced in epithelized pockets formed by the detachment and loss of supporting structure of the canine teeth.

2. This reattachment and regeneration occurred without removing the epithelium from the inner aspects of the soft tissue wall of the pocket.

3. The reattachment was produced by a deposition of new cementum on the denuded root surface.

4. Resorption of the old cementum and of some dentin occurred prior to the deposition of new cementum.

5. Following the regeneration of the lost alveolar process, the principal fibers of the periodontal membrane assumed their normal oblique arrangement.

The histological sections were prepared by Mr. G. Pudymaites, the photomicrographs by Mr. W. Wilson.

The author wishes to acknowledge his indebtedness to Drs. C. H. Best, A. W. Ham, and C. C. Lucas for their many helpful suggestions throughout the course of this investigation, and to Margaret Cornell and Joan Ashdown for their help in the preparation of the manuscript.

REFERENCES

1. Beckwith, T. D., Williams, A., and Fleming, E. C.: The Regeneration of Rodent Peridental Membrane, *Proc. Soc. Exper. Biol. & Med.* **24**: 562, 1927.
2. Beckwith, T. D., and Williams: Regeneration of Peridental Membrane in the Cat, *Proc. Soc. Exper. Biol & Med.* **25**: 713, 1928.
3. Beube, F. E.: A Study on Reattachment of the Supporting Structures of the Teeth, *J. Periodont.* **18**: 55, 1947.
4. Linghorne, W. J., and O'Connell, D. C.: Studies in the Regeneration and Reattachment of Supporting Structures of the Teeth. I. Soft Tissue Reattachment, *J. D. Res.* **29**: 419, 1950.
5. *Idem*: Studies in the Regeneration and Reattachment of Supporting Structures of the Teeth. II. Regeneration of Alveolar Process. *J. D. Res.* **30**: 604, 1951.
6. *Idem:* Studies in the Reattachment and Regeneration of the Supporting Structures of the Teeth. III. Regeneration in Epithelized Pockets, *J. D. Res.* **34**: 164-177, 1955.

THE INFLUENCE OF DIETARY THYROID ON THE BONES AND PERIODONTIUM OF RATS ON TOTAL AND PARTIAL TRYPTOPHAN DEFICIENCIES

L. A. BAVETTA, S. BERNICK, AND B. ERSHOFF

Department of Biochemistry and Nutrition, School of Dentistry and Medicine, and Department of Anatomy, School of Medicine, University of Southern California, Los Angeles, Calif.

IT IS becoming increasingly evident that requirements for specific nutrients are not static in the animal organism. Any factor that interferes with the digestion, absorption, or utilization of nutrients, or increases their destruction or excretion may result in malnutrition despite the apparent adequacy of the diet fed. Body requirements for essential nutrients may furthermore be significantly increased for purposes of detoxification or by factors such as physical exertion, fever, drugs, toxins, burns, fractures, and other trauma, major surgical procedures, abnormal environmental conditions and others. The net result of such factors is an increased body requirement beyond the usual or average range and the precipitation of nutritional deficiencies on diets that would otherwise be adequate. The induction of hyperthyroidism by the feeding of thyroactive substances has been widely employed as an experimental tool for the production or accentuation of nutritional deficiencies. By means of this technic an increased requirement has been demonstrated not only for some of the water-soluble and fat-soluble vitamins, but for dietary fat and certain minerals and amino acids as well. The effects of tryptophan deficiency on the bones and dental structures of rats have been described by Bavetta and Bernick in an earlier report.[1] In the present communication, data are presented on the effects of experimental hyperthyroidism on the bones and dental structures of rats fed a tryptophan-deficient diet and the effects thereon of graded levels of dl-tryptophan supplementation.

MATERIALS AND METHODS

Two basal rations were employed in the present experiment, Diet A and Diet B. Diet A was a tryptophan-free ration of the following composition: sucrose, 61 per cent; acid hydrolyzed casein, 24 per cent; cottonseed oil (Wesson), 10 per cent; and salt mixture, 5 per cent. To each kilogram of the above were added the following crystalline vitamins: thiamine hydrochloride, 40 mg.; riboflavin, 20 mg.; pyridoxine hydrochloride, 20 mg.; calcium pantothenate, 100 mg.; nicotinic acid, 100 mg.; ascorbic acid, 200 mg.; biotin, 4 mg.; folic acid, 10 mg.; para-aminobenzoic acid, 400 mg.; inositol, 800 mg.; vitamin B_{12}, 150 mcg.; 2-methyl-naphthoquinone, 6 mg.; choline chloride, 2 Gm.;

Supported by the Office of Naval Research, Department of Navy O.N.R. 228(08), National Institute for Dental Research, National Institutes of Health (D-194) and the Southern California State Dental Association.

Received for publication Aug. 23, 1955.

vitamin A, 5,000 U.S.P. units; vitamin D_2, 500 U.S.P. units; and alpha tocopherol acetate, 100 mg. Diet B was similar in composition but contained 0.5 per cent U.S.P. desiccated thyroid. The vitamins and desiccated thyroid were added in place of an equal amount of sucrose. In addition to the basal rations the following diets were also employed, consisting of basal rations A and B plus each of the following supplements: (a) 600 mg. dl-tryptophan per kilogram of diet, (b) 1.2 Gm. dl-tryptophan per kilogram of diet, and (c) 2.4 Gm. dl-tryptophan per kilogram of diet. These supplements were incorporated in the diet in place of an equal amount of hydrolysate and corresponded to 0.25, 0.50, and 1.00 per cent, respectively, of the hydrolysate content of the basal ration. In addition to the above diets, experiments were also conducted with diets which were identical to the basal rations A and B except that the acid hydrolysate were replaced by intact casein.

One hundred albino female rats of the University of Southern California strain were selected at 21 to 24 days of age and an average body weight of 49 Gm. (range 36 to 50 Gm.) for the present experiment. Animals were placed in metal cages with raised screen bottoms and were feed the above diets ad libitum (10 rats per group). Diets were made up weekly and stored under refrigeration when not in use. Rats were fed daily, and all food not consumed 24 hours after feeding was discarded. These measures were employed to minimize oxidative changes in the diet. After 28 days of feeding, surviving animals were sacrificed and their heads and long bones were removed and fixed immediately in Bouin's solution. The blocks of tissue were decalcified in 10 per cent nitric acid and in 10 per cent formalin. The specimens were then prepared for nitrocellulose embedding by the routine manner. The blocks of tissue were sectioned 20 to 30 microns thick and stained with hematoxylin and eosin.

OBSERVATIONS

Growth.—The experimental growth curves are seen in Fig. 1. The growth increment was directly related to the degree of supplementation with dl-tryptophan. The animals receiving 1.0 per cent dl-tryptophan attained the greatest growth whereas the other groups showed a decrease in their rate of growth. The growth increment of all the groups of animals receiving 0.5 per cent thyroid extract was depressed as compared to comparable groups not receiving thyroid; in fact, the animals existing on tryptophan deficient diet failed to survive the experimental period. The weight increase on hydrolysate was less than on intact casein.

Long Bones.—The effect of total absence of tryptophan from the diet on chondrogenesis and osteogenesis in the proximal epiphyseal plate is seen in Fig. 2. There is a marked reduction in the width of the epiphyseal plate as a result of a decrease in the size and numbers of cells of the different zones of the cartilage. At the same time there appears a relative increase of the intercellular matrix. A thin layer of lamellar bone is being deposited at the diaphyseal border thus prematurely sealing off the cartilage from the diaphysis and preventing the fingerlike extensions of the calcified cartilagenous matrix from protruding into the diaphysis. There is also loss of trabeculae in the epiphysis

and diaphysis. However, only a very slight narrowing of the epiphyseal plate is produced in animals subsisting on a marginal diet of 0.25 per cent tryptophan. This has been achieved primarily as a result of a decrease in the number of cells present in all the layers of the cartilage. In contrast to the previous figure there is no indication of osteoporosis in both the diaphysis and epiphysis (Fig. 3).

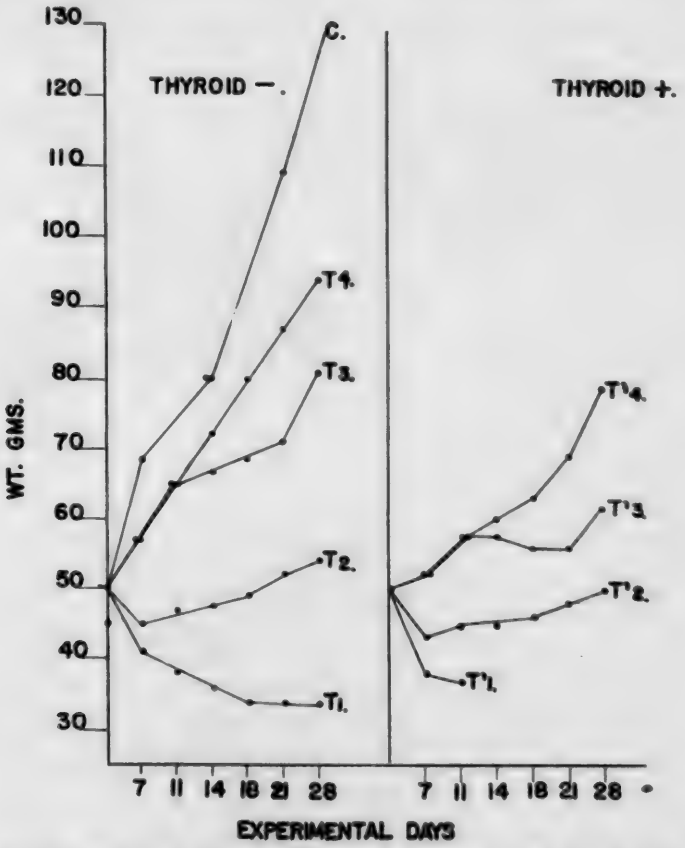
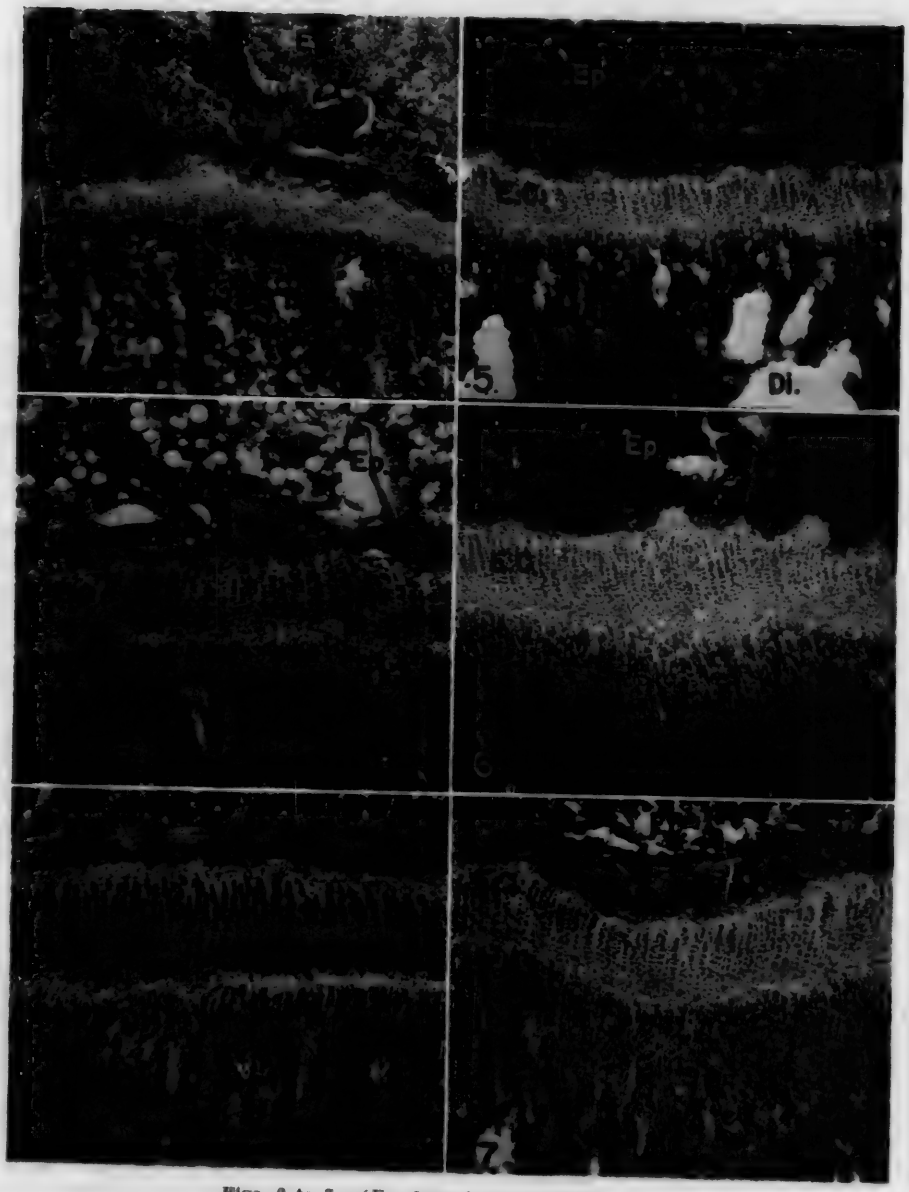


Fig. 1.—The influence of extra dietary thyroid on body weights of rats fed intact casein as well as casein hydrolysates supplemented with various levels of dl-tryptophan. *C.*, intact casein diet. *T4.*, 1.0 per cent tryptophan supplementation. *T3.*, 0.5 per cent tryptophan supplementation. *T2.*, 0.25 per cent tryptophan supplementation. *T1.*, Tryptophan-free diet. *T'4.*, 1.0 per cent tryptophan plus 0.5 per cent thyroid supplementation. *T'3.*, 0.5 per cent tryptophan plus 0.5 per cent thyroid supplementation. *T'2.*, 0.25 per cent tryptophan plus 0.5 per cent thyroid supplementation. *T'1.*, Tryptophan-free plus 0.35 per cent thyroid supplementation.

Animals that had received either 0.5 or 1.0 per cent tryptophan added to the basic deficient diet did not show any degenerative alterations to the epiphyseal area. The width and nature of the epiphyseal cartilage in both cases appeared normal for animals of this age group. Fig. 4 represents a section from an animal that had received 1 per cent tryptophan supplementation showing the active chondrogenesis and osteogenesis.



Figs. 2 to 7.—(For legend, see opposite page.)

The addition of thyroid extract to the diet results in some slight but definite changes. The animals that were placed on the tryptophan-free diet plus thyroid could not withstand the added stress and all of the experimental animals of this group died before the termination of the experiment. On the other hand, animals receiving 0.25 per cent tryptophan plus thyroid survived the experimental period. These animals showed a slight narrowing of the epiphyseal plate as wel as a marked loss of trabeculation in both the diaphysis and epiphysis (Fig. 5).

The epiphyseal plate of animals receiving 0.5 per cent tryptophan and thyroid appears slightly narrowed, however the main difference between these animals and its controls (Fig. 4) is the loss of trabeculation in the epiphysis and diaphysis (Fig. 6). The addition of adequate amounts of tryptophan in the form of 1.0 per cent tryptophan supplementation appears to be capable of overcoming the above histologic alterations in the normal development of bone (Fig. 7).

Alveolar Bone.—The periodontium of the molar teeth of animals placed on the tryptophan-free diet shows intense degeneration (Fig. 8). Not only is there osteoporosis of the spongiosa of the interradicular region but fragmentation of the cribriform plate by osteoclastic action may be observed. There is a loss of myeloid tissue and a replacement by proliferating connective tissue. The trabeculae that remain in the cribriform plate shows signs of osteogenesis as osteoid tissue is present, lining the spicules of bone. The marrow space of the interseptal bone has enlarged with a partial oblique destruction of its crest resulting in a decreased height. The transseptal and crestal fibers are disorganized and are replaced by proliferating connective tissue and inflammatory cells. The interproximal gingiva is undergoing inflammatory changes characterized by the heavy infiltration of chronic inflammatory cells in both the epithelium and subepithelial connective tissue.

The animals that received the 0.25 per cent tryptophan to their diet exhibited lesser degenerative changes to the periodontium. These animals show osteoporosis of both the interradicular and interseptal areas (Fig. 9). The gingival appearance is quite normal, however there is a proliferation of the

---

Figs. 2 to 7.—Photomicrographs of sections of proximal epiphyseal region of the tibia. *Ca.*, calculus. *C.T.*, proliferating connective tissue. *D.*, dentin. *Di.*, diaphysis. *E.C.*, epiphyseal cartilage. *Ep.*, epiphysis. *G.*, gingiva. *Ir.*, interradicular region. *Is.*, interseptal region. *Pu.*, pulp. (Hematoxylin and triosin, ×250; reduced ¾.)

Fig. 2.—Experimental animal placed on a tryptophan-free diet. Note the narrowing of the epiphyseal cartilage, and the loss of trabeculation in the epiphysis and diaphysis.

Fig. 3.—Experimental animal placed on 0.25 per cent tryptophan-supplemented diet. The thickness and characteristics of the epiphyseal plate appears normal, however there is slight loss in cell size and numbers in all the layers of the plate.

Fig. 4.—Experimental animal placed on 1.0 per cent tryptophan-supplemented diet. Note the well-organized zones of the epiphyseal cartilage, and the well-developed trabeculae in the epiphysis and diaphysis.

Fig. 5.—Experimental animal placed on a 0.25 per cent tryptophan and 0.5 per cent thyroid-supplemented diet. Observe the osteoporosis in both the diaphysis and epiphysis.

Fig. 6.—Experimental animal placed on a 0.5 per cent tryptophan and 0.5 per cent thyroid-supplemented diet. Compare with *Fig.* 4. Note the narrower epiphyseal cartilage and loss of trabeculations in the epiphysis and diaphysis.

Fig. 7.—Experimental animal placed on a 1.0 per cent tryptophan and 0.5 per cent thyroid-supplemented diet. This section differs from that of *Fig.* 4 by the slight narrowing of the epiphyseal plate and loss of bone in the epiphysis and diaphysis.

Figs. 8 to 13.—Mesiodistal sections of the upper first and second molar region. For key to abbreviations, see legend to Figs. 2 to 7. (Hematoxylin and triosin, ×250; reduced ⅝.)

Fig. 8.—Animal maintained on a tryptophan-free diet. Note the extreme osteoporosis in the spongiosa of the interradicular bone, and replacement with connective tissue. The gingiva exhibits infiltration by chronic inflammatory cells.

Fig. 9.—Animal maintained on a 0.25 per cent tryptophan-supplementation diet. Note the slight osteoporosis in the interradicular and interseptal bone.

Fig. 10.—Animal maintained on a 1.0 per cent tryptophan-supplemented diet. The alveolar bone, periodontal membrane, and gingiva appear normal.

Fig. 11.—Animal maintained on a 0.25 per cent tryptophan and 0.5 per cent thyroid supplementation. Not only is there loss of bone in the interradicular area, but only a sliver of bone remains in the interseptal region. Observe severe destruction of the gingiva.

Fig. 12.—Animal maintained on a 0.5 per cent tryptophan and 0.5 per cent thyroid supplementation. Note the severe damage of both the interseptal bone and overlying gingiva.

Fig. 13.—Animal maintained on a 1.0 per cent tryptophan and 0.5 per cent thyroid supplementation. Observe the slight osteoporosis in interradicular and interseptal bones.

basal layer of the epithelium and a few inflammatory cells in the subepithelial connective tissue. When 0.5 and 1.0 per cent tryptophan is added to the basic deficient diet, the response of the alveolar bone is striking for, as these levels, the bone, periodontal membrane, and gingiva appear normal (Fig. 10).

However, animals with 0.5 per cent desiccated thyroid added to the diet presented a somewhat different response. It appears that this introduces another stressing agent to the diet and aggravates the histologic disturbances. As previously mentioned, animals on tryptophan-free diet failed to survive the experimental period. The reaction of the periodontium in animals receiving 0.25 per cent tryptophan plus thyroid is seen in Fig. 11. An osteoporosis of the interradicular spongiosa is observed, however the marrow spaces are filled with proliferating connective tissue. The greatest damage is observed in the interseptal bone where only a thin sliver of bone remains. In this region the attachment fibers are disorganized with the collagenous fibers interlacing in all directions. The interproximal gingiva displays severe degenerative changes. Calculus is present in the interproximal space resulting in the complete ulcerations of the surface epithelium. In addition, there is a recession of the epithelial attachment onto the cementum. The comparable findings in animals receiving 0.5 per cent tryptophan and thyroid is seen in Fig. 12. These results are to be compared directly with those shown in Fig. 10. It is apparent that at the same levels of tryptophan supplementation much more severe damage is imposed on the periodontium as a result of thyroid activity. There is bone loss in the interradicular region but not as damaging as in Fig. 11. The bone marrow spaces are filled with loose connective tissue and the attachment fibers are still well organized. However, in this case the damage to the interseptal bone is also severe. This is exemplified by an increase in the size of the marrow spaces, an osteoclastic destruction of the crest of the bone, and infiltration of the marrow spaces by proliferating connective tissue. There is calculus present and due to its pressure it had produced a crater-shaped gingiva. The surface epithelium is ulcerated and the inflammatory cells have infiltrated into the epithelium. The animals that were given both thyroid and 1.0 per cent tryptophan to the diet showed minimal changes to the periodontium of the molar teeth. There is slight osteoporosis of the interradicular and interseptal bone. The gingiva in this situation appear normal (Fig. 13).

DISCUSSION

The present investigation suggests that the tryptophan requirement of rats is increased in the hyperthyroid animal. This is evidenced by the fact that desiccated thyroid, under the conditions of the present experiment, accentuated the pathologic changes obtained on tryptophan-free rations.

Whereas the addition of 600 mg. dl-tryptophan per kilogram of diet largely corrected the pathologic changes in the bones and dental structures of rats on a tryptophan-free ration in the absence of dietary thyroid, this level of tryptophan supplementation failed to correct these changes when dietary thyroid was present. Higher levels of tryptophan supplementation resulted in minimal pathologic changes in bones and dental structures in both series.

The present findings suggest that diets which may be relatively adequate for the development of bone and dental structures may become inadequate under varying conditions of stress.

SUMMARY

The effects of experimental hyperthyroidism were studied on the bones and dental structures of rats fed a tryptophan-deficient ration supplemented with graded levels of dl-tryptophan. Diets containing 600 mg. dl-tryptophan per kilogram of diet or less resulted in inhibition and retardation of both endochondral and periosteal bone formation. Marked osteoporosis was noted in the alveolar bone of the molar teeth.

These effects were accentuated by desiccated thyroid. At higher levels of tryptophan supplementation, the above findings were minimal in both control and thyroid-fed groups. Ulceration of gingival epithelium and crater formation occurred in rats fed a tryptophan-free ration supplemented with 600 mg. or less of tryptophan in animals receiving dietary thyroid. These findings were not observed in animals receiving identical rations in the absence of dietary thyroid.

REFERENCE

1. Bavetta, L. A., and Bernick, S.: The Effect of Tryptophan Deficiency Upon the Jaws of Rats, *J. Dent. Res.* 33: 309-315, 1954.

TOOTH CONTACT DURING CHEWING

D. J. ANDERSON, PH.D., M.SC., B.D.S., AND D. C. A. PICTON, B.D.S.

Physiological Laboratory and Dental Department, Guy's Hospital, London, England

INTRODUCTION

THE main justification for the treatment known as bite rehabilitation or occlusal equilibration appears to be the assumption that the teeth come into centric occlusal contact during chewing. If this occurs and if the occlusion is normal, the masticatory loads are distributed (not necessarily equally) throughout the whole dental arch. If the teeth do not come into contact during chewing, but remain separated by food, then occlusal equilibration seems to be justified only in gross malposition of the teeth, or in individuals who indulge in bruxism.

The problem of occlusal contact during chewing has been investigated by only a few workers. Hildebrand,[1] using radiographic technics, concluded that at the commencement of the chewing movement there is slight lateral gliding of the lower denture against the upper, and at the termination of the movement "the mandible reaches the occlusal position after eventual gliding of the lower denture against the upper." Jankelson, Hoffman and Hendron[2] reported results obtained by completing an electrical circuit between opposing teeth, which showed that contact during chewing did not occur or was insignificant. In some instances it appeared at the first swallow, in others only during the second or third swallow. The record shown in their paper was obtained from one subject eating apple. Yurkstas and Emerson,[3] using a similar technic on 12 subjects with artificial dentures showed a surprising frequency of tooth contact both on the working and balancing sides. The subjects chewed meat and bread sandwiches.

The series of experiments to be reported here was designed to investigate the frequency of tooth contact in the natural dentition during chewing. Ten male subjects of ages ranging between 20 and 34 years provided the experimental data. All except one (Subject 1) were student volunteers, who were not chosen on the basis of their dental condition; the only requirement being the presence of amalgam restorations in one pair of opposing posterior teeth. In all cases the dentition was complete or nearly so.

METHOD

It was decided to record contact between the teeth by completing an electrical circuit through them using a small voltage applied to the circuit to deflect one beam of a double-beam oscilloscope. In order to relate the frequency of contact to the number of chewing movements, electromyographic

Received for publication July 13, 1955.

records from one of the masseter muscles were obtained on the other oscilloscope beam. Fine gauge silver or stainless-steel wires were embedded in amalgam in existing tooth cavities and were carried on to the buccal surface of the teeth without interfering with normal occlusion. In some cases an additional wire was let into a suitable part of the occlusal surface of one of the existing amalgams if the wires on the buccal surfaces failed to make a

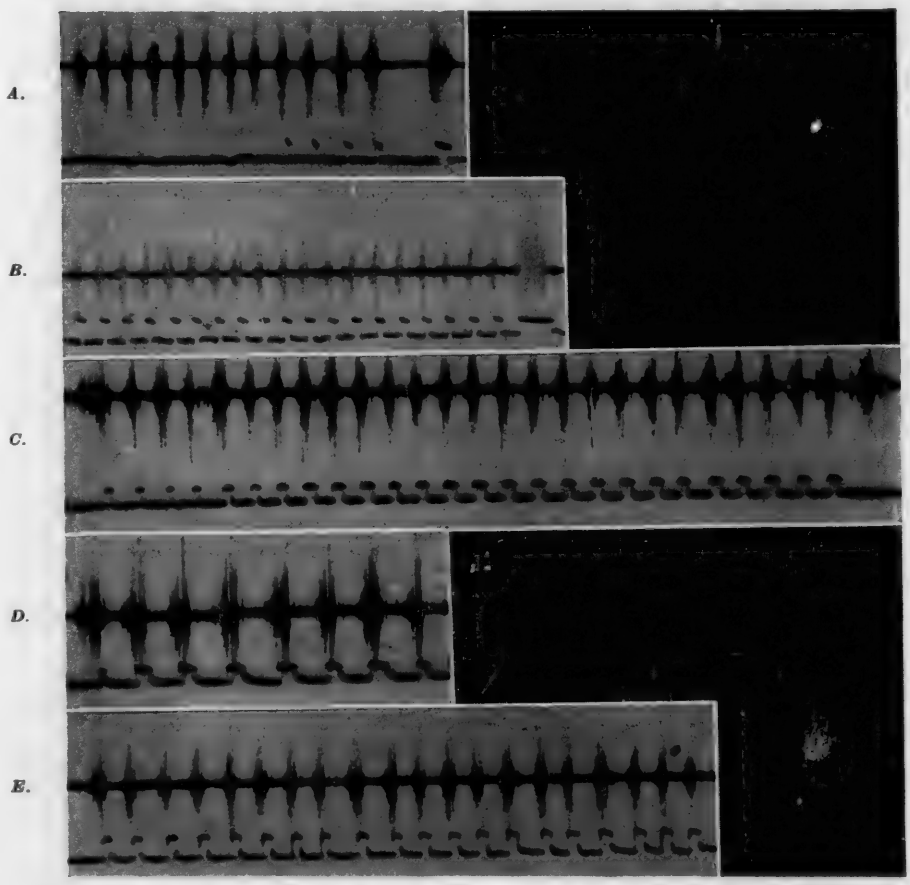


Fig. 1.—Oscilloscope records of electrical activity in the masseter on the chewing side (upper tracing) and contact between the teeth on the opposite side. Tooth contact is shown by an elevation of the lower tracing. *A*, Subject 1. Biscuit; *B*, Subject 2. Carrot; *C*, Subject 6. Apple; *D*, Subject 8. Bread; *E*, Subject 10. Meat.

satisfactory contact after adjustment. In every case great care was taken to ensure that the wires allowed unhindered movement and closure of the jaws. If an occlusal wire was necessary, a slot was provided for it in the amalgam so that it did not interfere with occlusion.

When an experiment was contemplated, fine insulated leads were attached to the buccal extensions of the wires embedded in the teeth and passed out of

the mouth to complete the rest of the circuit. Electromyographic records were taken with surface electrodes applied by suction to the skin over the masseter, and the potentials obtained were suitably amplified. The tooth wires were carefully adjusted to make contact with the teeth in centric occlusion only; the slightest lateral movement breaking the circuit. This was checked after every chewing sequence. The critical nature of the contact and the fineness of the leads from the chosen teeth made it necessary for the records to be taken with the subject chewing on the opposite side of the mouth. The electromyographic records were taken from the chewing side.

Five food materials were chosen for the experiments. These were: shortbread biscuit, raw carrot, apple, cooked meat, and crustless bread. They were given in 0.5 inch cubes and the subjects were instructed to chew them without incisor biting and to give a signal as soon as the material had been swallowed.

RESULTS

Typical records are shown in Fig. 1. In most subjects contact was least frequent with biscuit, none of them making contact on every thrust. With the other materials, most subjects brought their teeth into contact for more than one-half the thrusts, some for all the thrusts with every material. When contact occurred it was always more frequent toward the end of the chewing sequence. The results are shown graphically in Fig. 2, the number with each point referring to a particular subject. The means and standard deviations of the data for each subject were computed and are available from the authors.

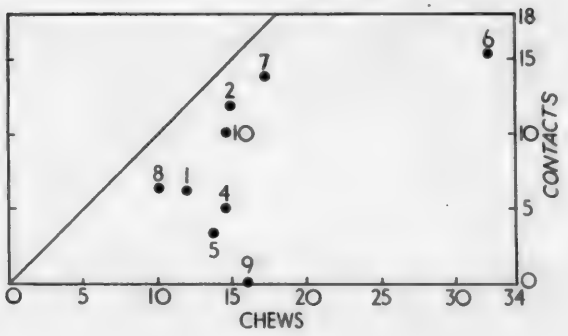
It will be observed that Subject 6 executes many more chewing movements for every material than the other subjects. There was no gross dental abnormality which might be held to account for this. His arches were complete save for the loss of $\frac{|4}{5|}$ and the occlusion was only a mild example of Angle's Class II, division 1.

The frequency of contact in this series is surprisingly high, and in only 2 of the subjects were consistently low values recorded with all materials. It is noteworthy that the duration of each contact increased toward the end of the chewing sequence.

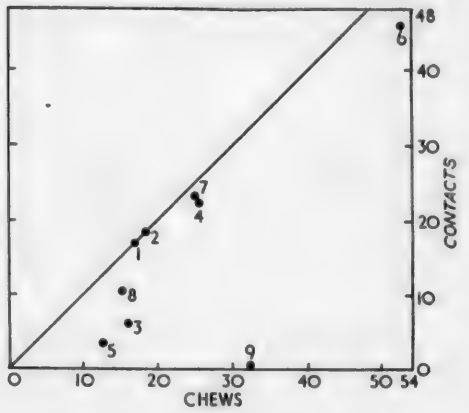
DISCUSSION

The object of the experiments reported here was to determine the frequency of contact between the dental arches during chewing, contact being registered with the teeth in centric occlusion. The wire used to complete the electrical circuit between the opposing teeth was approximately 0.5 mm. in diameter and in all cases the smallest possible voluntary gliding movement with the teeth in contact broke the circuit. In the empty mouth the method therefore records centric occlusal contact and an extremely narrow range of gliding movements out of centric occlusion.

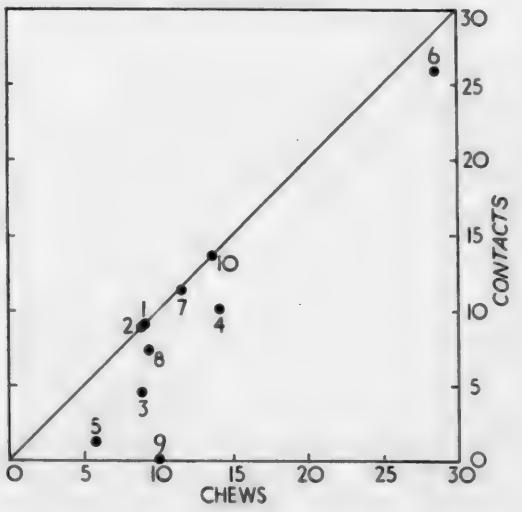
During chewing, the food was on the side opposite to that used for recording contact and it is therefore possible that the teeth may have been separated



A.



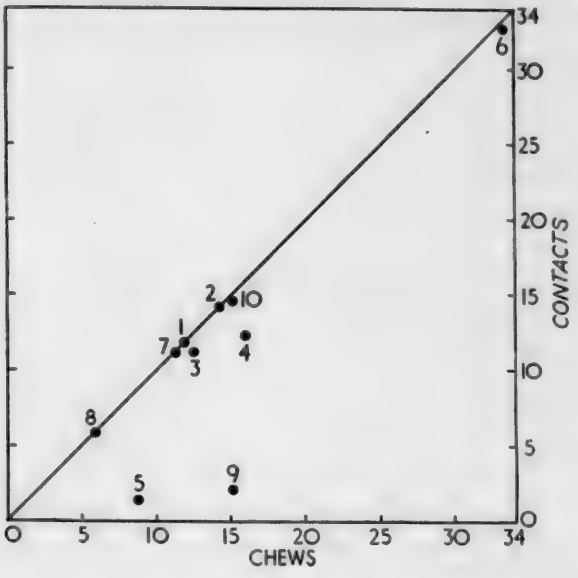
B.



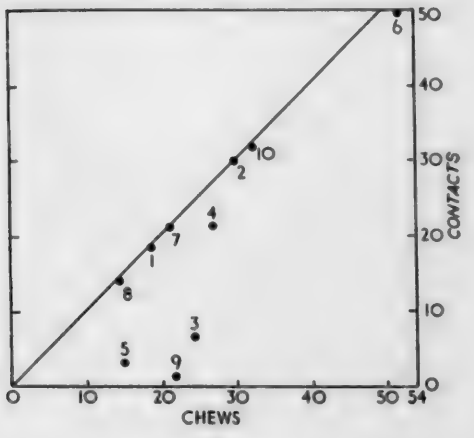
C.

Fig. 2.—The relation between the number of chewing movements and the number of contacts. The sloping line shows the direction which would be taken by the points if contact occurred with every chewing movement. The number with each point identifies the subject. *A*, biscuit; *B*, carrot; *C*, apple.

by food on one side while coming into contact on the other. This is considered unlikely for several reasons. Attempts by the subjects to break the electrical contact while keeping the teeth together on the other side always



D.



E.

Fig. 2.—Cont'd. *D*, bread; *E*, meat.

failed. This shows that the jaws are not easily tilted. In some of the subjects, separation of the teeth on the chewing side with paper 0.0015 inch thick broke the contact on the other side, while in others paper twice as thick was necessary. This shows that although tilting can occur it is only very slight. If it is conceded that contact can be recorded on one side while the other side

is slightly separated, then during chewing, this separation would have to be maintained in spite of the fact that the main muscular effort tended to overcome it. It seems unlikely that any of the food materials used in this investigation, except biscuit, could be capable of this.

In view of the surprisingly high frequency of contact shown by most subjects, the teeth were examined for evidence of wear. This was most marked in Subject 1 who made contact on every thrust with all the foods except biscuit. This subject, aged 34, was approximately 12 years older than the others. Signs of considerable wear were seen in all the teeth, even the third molars, and the facets suggested that he indulged in nonfunctional grinding of the teeth. Most of the other subjects showed some attrition but it was not possible to grade it accurately. None of the subjects showed any gross orthodontic abnormalities, the occlusion conforming with Angle's Class I, or in some cases showing a tendency toward Class II or III. Subject 9, who brought his teeth into contact most infrequently of all the subjects, showed a considerable incisor overbite, the lower incisors touching the palate, having drifted back following the loss of $\overline{6|6}$. The upper incisors just came into contact with the gingiva in front of the lower incisors. The subject was not conscious of this fact.

SUMMARY

1. Contact between opposing teeth during chewing was recorded electrically in 10 subjects with 5 food materials.

2. The results confirm the hitherto unsupported assumption by many periodontologists that the teeth make contact during chewing.

3. The teeth came into contact for more than one half the chewing thrusts in most subjects. In some subjects every thrust made contact, but this was never the case with biscuit.

4. The results strongly suggest that when contact occurred it was centric occlusal contact over the entire arch.

Our thanks are due to the subjects for their co-operation and to the Nuffield Foundation for a grant to one of us (D. J. A.) which has supported this work.

REFERENCES

1. Hildebrand, G. Y.: Skand. Arch. Physiol., suppl., vol. 61., Studies in the Masticatory Movements of the Lower Jaw, 1931.
2. Jankelson, B., Hoffman, G. M., and Hendron, J. A.: *J. A. D. A.* 46: 375-386, 1953.
3. Yurkstas, A. A., and Emerson, W. H.: *J. Pros. Dent.* 4: 168-174, 1954.

TISSUE REACTION AROUND ACRYLIC ROOT TIPS

JENS WAERHAUG

The Institute of Dental Research, Oslo, Norway, and the Division of Periodontology, School of Dentistry, University of Minnesota, Minneapolis, Minn.

THE reaction of the soft tissue to porcelain root tips has been studied by Immenkamp[6] and Dewey and Zugsmith.[3] Immenkamp[6] believed that the artificial pocket between the root tip and the surrounding soft tissue would not be epithelized and that it represented a permanent path of infection. Dewey and Zugsmith[3] concluded, "The fact emphasized by several investigators that highly glazed porcelain is a non irritating substance, is brought out well by the findings in our implantation experiments." Discussing the pocket around root tips, they write furthermore: "A pocket about a natural root is not identical with the socket about a porcelain root when it is covered with epithelium. Bacteria in such an epithelized socket tend to develop local immunity of the underlying tissue in a measure comparable to that in other parts of the oral cavity. Conditions in a pathological pocket about a natural root are quite different. Here the tissue of the socket is in organic union or associated with the root structure." In the aforementioned experiments, the root tips were removed before sectioning. Thus, the border line between the implanted material and the soft tissue could not be studied. This technical problem was solved in an earlier experimental series on the tissue reaction around acrylic crowns.[8] By dissolving these crowns in chloroform instead of pulling them out, the soft tissue bordering upon the crowns was not torn or disarranged. The same principle has also been utilized by Zander[12] who inserted cellulose acetate films into healthy pockets in dogs in order to study the "epithelial attachment." A microscopic examination in incident light of root tips that had been in contact with the soft tissue for some time revealed that apparently normal epithelial cells could adhere to these root tips, thus indicating that the epithelium joined them in the same way as the epithelial cuff joins the tooth surface.[7] The purpose of the present investigation is to provide more information on the tissue reaction around root tips, information which may be of value in the construction of bridges. Furthermore, this study represents a link in a series of experiments that have been done in order to judge whether or not there is any basic difference between the adaption of the epithelium to inert foreign materials and the adaption of the "epithelial attachment" to natural teeth.

This investigation was supported by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

Received for publication Aug. 15, 1955.

MATERIAL AND METHOD

The experiment was undertaken on 13 teeth in 4 dogs and 10 teeth in 4 monkeys. Gold bridges with long acrylic root tips were made and the teeth to be replaced were extracted immediately prior to the insertion of the bridges. Five of the teeth fractured and it proved impossible to remove the roots. In spite of this the bridges were cemented, as healing under such circumstances might be of interest. The root tips which were well polished, reached 5 to 8 mm. below the gingival margin, and filled out the socket fairly well. They were allowed to remain in place for observation periods varying between 19 days and 338 days. The bridges in the 4 monkeys were thoroughly brushed once every day. All the animals were closely examined at regular intervals and notes on the clinical condition were recorded. At the end of the observation period the animals were decapitated under pentobarbital sodium anesthesia. The jaws were carefully removed and fixed in 10 per cent formalin for 2 days. They were then transferred to chloroform in which the acrylic root tips were dissolved.[8] Until this stage precautions were taken not to move the soft tissue in relation to the root tip in order to prevent artefacts. The acrylic pontics having been dissolved, the jaws were decalcified in 5 per cent nitric acid and the endpoint of decalcification checked by fluoroscopy.[11] The jaws were cut into blocks suited for sectioning after decalcification. During the whole preparation process the specimens were extremely carefully handled and washed so that the accumulations of deposits might not be washed away. Some of the specimens were embedded in paraffin, and the rest of them in celloidin. They were cut either in buccolingual, or in mesiodistal direction and the sections were stained with hematoxylin-eosin.

OBSERVATIONS

Clinically the healing was found to be satisfactory in the majority of cases. There was some recession of the gingival margin, mainly buccally, and sometimes a furrow was observed about 2 mm. below it; otherwise no conspicuous difference could be seen as compared with the natural teeth.

The histologic sections revealed a rather broad variation. After 19 days the healing was well advanced (Fig. 1), and there was only a slight inflammation. The epithelium had proliferated along the surface of the root tip, but had not as yet completely covered its apical portion. The pattern of the epithelium was normal, but polymorphonuclear leukocytes were usually found bordering the surface of the root tip. In most of the cases with observation periods longer than 78 days, the root tips were completely covered by epithelium. In 13 of these cases perfectly normal epithelial cells bordered directly upon a part or the whole of the root tip (Figs. 2, 4, 5, 7, 9, and 10). However, in a number of cases hyalin-like bodies were observed between the epithelial cells (Figs. 2 and 4). Degeneration of single cells within the epithelial cuff could occasionally be observed (Fig. 4, *D.e.*). In other cases the surface layer of the epithelium was degenerated (Fig. 3). Accumulations of supragingival bacterial plaque were generally observed in the interproximal spaces (Figs. 3,

Fig. 1.—Dog. Observation period 19 days. Acrylic root tip inserted in socket immediately after extraction. Slight inflammation in connective tissue. Upper ¾ of root tip covered by epithelium. Epithelial structure normal in deeper part, but bordering upon cuticle on surface of root tip are polymorphonuclear leukocytes, *PL*. Foreign bodies, *FB*, between epithelium and root tip.

Fig. 2.—Monkey. Observation period 338 days. Normal epithelial cuff adjusted to cuticle on surface of root tip (top right). Formation of hyalin bodies within epithelial cuff (bottom right). *HB*, accumulations of hyalin bodies. Infiltration of lymphocytes and plasma cells in connective tissue near gingival margin, decreasing toward apex of root tip.

4, 6, and 8) and in 11 cases (Table I) the plaque had grown apically along the surface of the root tip (Fig. 7, left). Where plaque was present, more or less severe inflammation of the connective tissue and degeneration of the epithelial cells were observed. Between the epithelium and the plaque, accumulations of exudate cells were usually present. Foreign bodies were often

Fig. 3.—Dog. Observation period 86 days. Epithelium, showing some degeneration, joins cuticle on surface of acrylic root tip. Little or no inflammation in connective tissue, except for area subjacent to plaque.

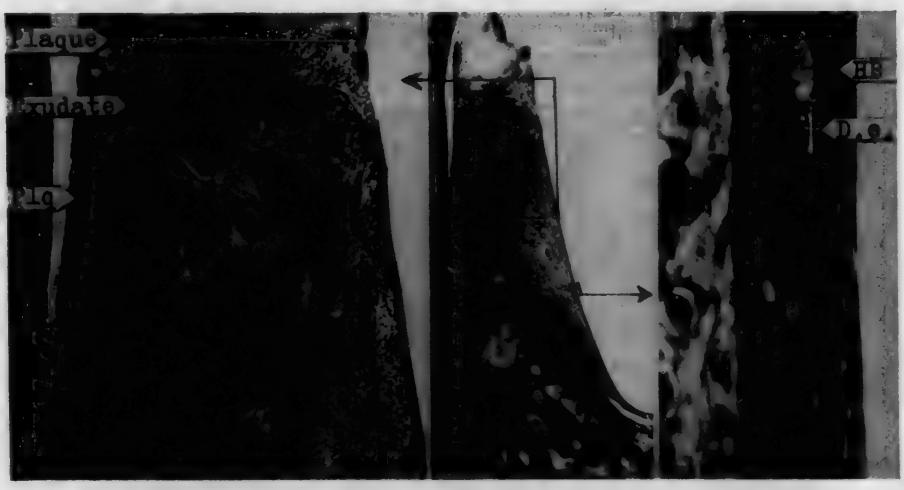


Fig. 4.—Monkey. Observation period 338 days. Normal epithelial cuff joins closely to cuticle on surface of acrylic root tip (right). Some degeneration of epithelial cells, *D.e.*, and formation of hyalin bodies, *HB*. Space between pontic and abutment tooth filled with bacterial plaque (*Plq*). Subjacent tissue heavily inflamed, decreasing toward apex of root tip.

seen between the epithelium and the root tip (Fig. 1, left), and in one case a rather large piece of bone was found completely embedded in the epithelial cuff of a pontic (Fig. 5). The bone cells were dead, but infection was apparently absent as the epithelium bordering upon the piece of bone showed little or no sign of irritation. In the 5 cases where the root had fractured during the extraction, remnants of the root or infected pieces of bone were found under the pontic (Fig. 8). In this particular case, the fractured root

Fig. 5.—The same specimen as Fig. 4, but different section. Higher magnification of epithelial cuff around acrylic root tip at level of alveolar crest. Piece of bone embedded in normal epithelial cells. Bone cells are dead. Inflammation in upper part associated with irritation caused by bacterial plaque.

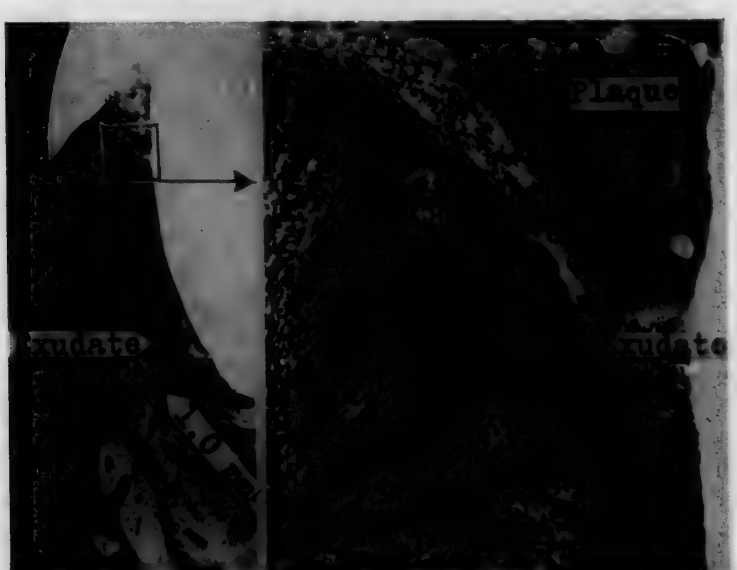


Fig. 6.—Dog. Observation period 86 days. Root fractured during extraction. Between epithelium and root tip is accumulation of exudate reaching toward apex of root tip. Space between root tip and abutment tooth filled with plaque in which are hairs. Heavy inflammation in connective tissue. Healing impaired by fractured root.

Fig. 7.—Monkey. Observation period 338 days. Root tip mainly covered by healthy epithelial cuff (bottom right) showing little or no difference from epithelial cuff of control tooth (top right). On left side, formation of subgingival plaque on surface of root tip. Epithelium thickened correspondingly to plaque, and exudate formed between. Alveolar wall resorbed corresponding to plaque.

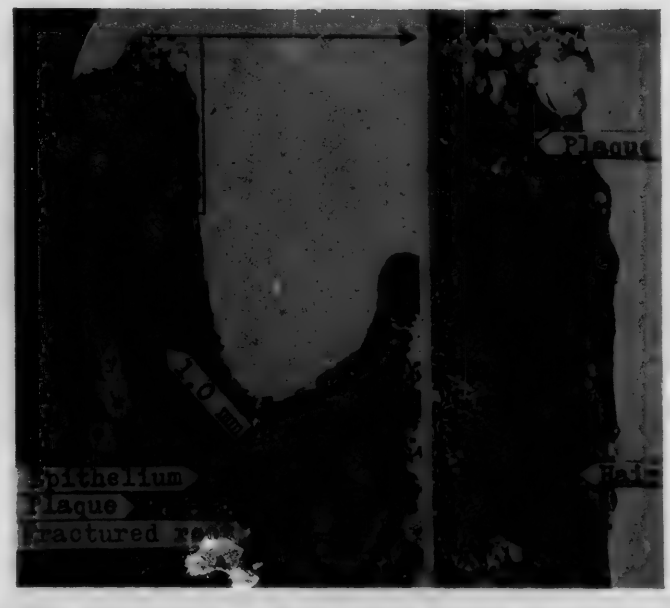


Fig. 8.—Dog. Observation period 86 days. Root fractured during extraction, and part left below root tip. Fractured root covered by bacterial plaque, surrounded by epithelium. Bone resorbed near to remnant of root. Supragingival plaque fills space between root tip and abutment tooth. Accumulations of exudate between root tip and epithelium.

was covered by a bacterial plaque and a severe inflammation was caused in the surrounding tissues. This shows the importance of a complete removal of the root under a pontic.

TABLE I. OBSERVATION PERIOD, PRESENCE (+) OR ABSENCE (−) OF NORMAL EPITHELIAL CUFF, DOWNGROWTH OF PLAQUE BELOW GINGIVAL MARGIN AND SMALLEST DISTANCE FROM BONE TO ROOT TIP IN THE 23 CASES REPORTED IN THIS EXPERIMENT

ANIMAL	TOOTH	OBSERVATION PERIOD (DAYS)	NORMAL EPITHELIAL CUFF	DOWNGROWTH OF PLAQUE (MM.)	DISTANCE BONE-ROOT TIP (MM.)
D 9	LL 3	19	−	0.0	0.4
D 10	LR 2	32	+	0.0	0.9
D 10	LL 3	32	−	0.0	1.1
D 10	UR 1	32	+	0.0	0.6
D 13	UR 5	86	+	0.7	0.5
D 13	UR 3	86	−*	3.0	1.0
D 13	UR 1	86	−*	2.0	1.0
D 13	UL 2	86	−*	3.0	0.6
D 13	UL 5	86	+	0.0	0.5
D 13	LR 3	86	+	0.0	1.0
D 13	LL 3	78	?*	0.0	0.6
D 14	UR 5	38	−	0.0	0.5
D 14	UL 3	20	−	0.0	0.6
M 2	UR 2	338	+	0.0	0.7
M 2	UL 2	338	+	0.5	1.1
M 4	LR 2	139	−	**	1.5
M 4	LL 2	139	−	**	1.6
M 5	UR 2	338	+	2.5	0.3
M 5	UL 2	338	+	0.4	0.4
M 5	LR 2	338	+	0.5	0.3
M 5	LL 2	338	+	0.0	0.4
M 6	LR 2	324	+	0.7	0.3
M 6	LL 2	324	+	0.0	0.3

? = disappeared during sectioning.
*Fractured during extraction.
**Completely covered with plaque.

In some cases where the specimens had been sectioned buccolingually, a furrow was observed some distance below the gingival margin (Fig. 9). The gingiva in such cases consisted mainly of circular fibers (Fig. 9, top left). However, in other cases the gingival margin looked just as it did around a natural tooth (Fig. 10).

In all cases where the epithelium bordered directly upon the root tip, a distinct cuticle was observed (Figs. 1 to 4, 7, 9, and 10). Formation of new bone in the socket adjacent to the root tip was apparent after 19 days (Fig. 1). In all cases there was a certain distance between the bone and the root tip (Figs. 1 to 4, 7, 9, and Table I). Where bacterial plaque or sequestra caused inflammation in soft tissue the distance was greater (Figs. 6 and 8 and Table I).

In Table I are tabulated the observation periods, the condition of the epithelium, the amount of downgrowth of plaque below the gingival margin and the distance from the bone to the root tip in the 23 cases under discussion.

DISCUSSION

This experimental series shows that an acrylic root tip is well tolerated by the soft tissue. In certain areas the epithelium joining the root tip had

Fig. 9.—Monkey. Observation period 338 days. Buccolingual section. Furrow located 3.00 mm. below gingival margin. Gingiva above furrow, consisting mainly of cross-cut circular fibers, poorly supported (top left).

Fig. 10.—Dog. Observation period 86 days. Normal epithelial cuff adheres to root tip to gingival margin. Slight infiltration of round-cells near gingival margin, decreasing to none in deepest part. Connective tissue fibers mainly arranged parallel to long axes or root tip.

the typical appearance of an "epithelial attachment" (Figs. 2, 4, 7, 9, and 10), and compared with real "epithelial attachments" from control teeth (Fig. 7, top right) little or no difference could be observed. However, a very slight irritation may be present, accounting for some degeneration of the epithelium (Fig. 3) and the peculiar formation of hyalin bodies (Figs. 2 and 4). These hyalin bodies, which apparently are homogenized epithelial cells, were rather commonly found in the epithelial cuff around the root tips in the monkeys, but were never observed in the epithelial cuff around natural teeth. The irritation, if any, must, however, have been very weak as the migration of exudate cells through the epithelium was slight, the appearance of such cells indicating irritation. Therefore, acrylic resin may be considered inert, or at least very close to inert, in relation to epithelium. This is in accordance with earlier observations on the tissue reaction around acrylic crowns.[8] Consequently, acrylic resin should be suited for crowns and root tips ar far as the tissue reaction is concerned.

Under favorable conditions the pocket around a root tip is comparable to a normal pocket where the epithelial cuff (epithelial attachment) adheres to the tooth surface all the way to the gingival margin, or close to it (Figs. 1 to 4, 7, and 10). As around natural teeth, bacterial plaque may be found above the gingival margin and such plaque invariably will cause a severe inflammation of the underlying soft tissue (Figs. 3, 4, 6, and 8). As around natural teeth, the bacterial plaque may also migrate below the gingival margin and cause an extensive inflammation there (Fig. 7). Therefore, preventing the formation of bacterial plaque on the pontic must be of prime importance. Bucally and lingually this can be done by means of a tooth brush (Fig. 10), but the toothbrushing does not remove the bacterial plaque in the interproximal spaces. This is apparent from Figs. 4 and 7, both pontics having been throughly brushed. For the cleansing of the interproximal spaces, tooth picks seem to be indispensible. The construction of the root tip may also facilitate the retension of bacterial plaque to various degrees. An interproximal space of the type shown to the right in Fig. 11 will necessarily be easier to keep clean than that to the left. In the right one the epithelium may even join directly to the bridge material.

The conditions in the interproximal areas differ to a certain extent from those in the buccal and linqual areas. Interproximally, the soft tissue around the root tips is supported by the adjacent teeth, while in the lingual and even more so in the buccal area, the soft tissue lacks firm support. Therefore, pictures like the one shown in Fig. 9 were commonly seen. This flabby margin is only loosely kept in contact with the root tip by means of the circular fibers[1] from which it is mainly built up. If the root tip is too long, the unsupported area will be too wide, and foreign bodies may be pressed down more easily into the pocket. Therefore, a root tip should be made as short as possible.

In Figs. 2, 3, 4, 7, and 9 and in Table I it can be seen that the distance from the alveolar bone to the root tip varies between 0.3 and 1.1 mm. in the areas where bacterial plaque is not present. This contour of the bone has arisen partly through resorption in areas where the root tip touched the bone

at the time of insertion, and partly through apposition in the socket. Consequently, there seems to be a factor which determines that bone cannot be formed closer than about 0.3 mm. to the surface of a root tip. On the other hand there is a tendency of the bone to be built up to that distance. This observation may have practical value for the construction of the root tips. These should not be placed so near to the abutment teeth that the entire interdental septum will be removed through the ensuing resorption. This mistake was made in most cases on the monkeys and is undoubtedly responsible for the rather low level of the alveolar crest in relation to the deepest point of the epithelium on the abutment teeth. Therefore, a construction as illustrated in the left interdental space in Fig. 11 should be avoided.

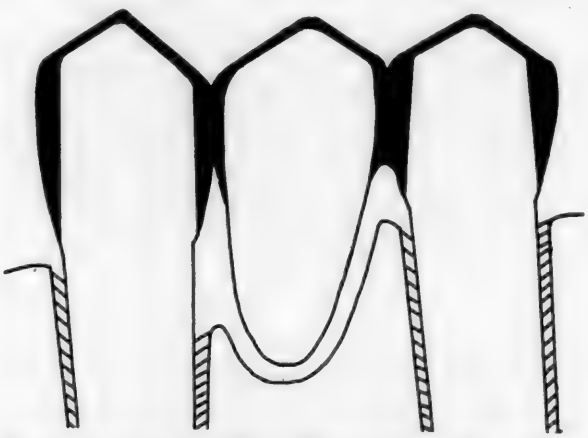


Fig. 11.—Construction of left interdental space is wrong: space between crown and pontic ending in knife edge. Root tip too close to abutment tooth, thus reducing height of alveolar crest. Right interdental space correctly constructed: space between crown and root tip rounded and root tip kept at a distance from abutment tooth.

Where bacterial plaque covered the root tip, the distance from this to the alveolar bone invariably was greater, demonstrating how inflammation of the soft tissue causes resorption of bone (Figs. 7 and 8). It is important to prevent the formation of bacterial plaque on root tips but, if supra- and subgingival plaque have been formed, normal conditions will most likely be reestablished if the plaque is thoroughly removed. This is in accordance with earlier observations concerning the effect of removal of subgingival calculus in pockets around natural teeth.[9]

It was interesting to note that the thin membrane which regularly was observed between the root tip and the adjacent healthy epithelial cells (Figs. 1, 2, 4, and 7) looked exactly like the "primary enamel cuticle" as it appeared in the "epithelial attachment" on control teeth (Fig. 7). This finding corroborates earlier observations concerning the "enamel cuticle," indicating that this may not be calcified and not necessarily formed by the ameloblasts.[8, 10]

The tissue reaction described about implanted root tips refers only to those made of acrylic resin. It cannot be concluded that the reaction is the same in human beings as it is in dogs and monkeys, even if no basic difference should be expected.

CONCLUSIONS

1. Heat-cured acrylic root tips may, under otherwise favorable conditions, be well tolerated by the periodontal tissues.

2. An epithelial cuff comparable to a normal "epithelial attachment" usually will be formed around the root tip, and inflammation may be absent.

3. Bacterial plaque may be formed on the root tip, both above and below the gingival margin, and cause an inflammation in the surrounding connective tissue.

4. There seems to be no basic difference between the pocket around an acrylic root tip and the pocket around a natural tooth.

5. After insertion of an acrylic root tip, the alveolar bone will be resorbed or rebuilt to a distance of 0.3 to 0.9 mm. from the root tip.

6. Acrylic root tips should not be made too long because too wide a rim of soft tissue without bony support may not be kept in sufficiently close contact with the tooth, thus allowing bacteria and foreign bodies to be pressed into the pocket.

7. Long acrylic root tips should not be placed too near to the abutment tooth, as the height of the interdental septum may be reduced.

8. A membrane similar to the "primary cuticle" observed on the epithelial cuff around acrylic root tips indicates that the present-day opinion about the "primary cuticle" may have to be revised.

9. The root under a pontic should be completely removed, as it most likely will cause a more or less severe inflammation.

SUMMARY

In the sockets of 13 teeth in 4 dogs and 10 teeth in 4 monkeys acrylic root tips fixed to bridges were inserted. Histologic observation after 19 days to 338 days revealed that the acrylic root tips were well tolerated by the soft tissue. In the majority of cases, the epithelium adhered closely to the root tip in the same way as the epithelial cuff (epithelial attachment) adheres to a natural tooth, and no inflammation was observed. However, in some cases the root tips were covered by bacterial plaque that caused more or less severe inflammation. A membrane similar to the "primary cuticle" was observed on the surface of the epithelial cuff around the acrylic root tips. In cases where the root had been fractured during the extraction, the remnants caused a severe inflammation under the root tip.

REFERENCES

1. Arnim, S. S., and Hagerman, D. A.: Connective Tissue Fibers of the Marginal Gingiva, *J. A. D. A.* **47**: 271, 1953.
2. Baume, L. J.: Structure of the Epithelial Attachment Revealed by Phase Contrast Microscopy, *J. Periodont.* **24**: 99, 1953.

3. Dewey, K., and Zugsmith, R.: An Experimental Study of Tissue Reactions About Porcelain Roots, *J. D. Res.* **13**: 459, 1933.
4. Gottlieb, B.: Der Epithelansatz am Zahne, *Dtsch. Monatsch. f. Zhk.* **39**: 142, 1921.
5. *Idem:* Ätiologie und profülaxe der Zahnkaries, *Ztschr. f. Stomat.* **19**: 129, 1921.
6. Immenkamp, A.: Ein Beitrag zur Klärung einiger Streitfragen in der keramischen Protetik auf Grund histologischer Untersuchingen, *Vjschr. f. Zhk.* **48**: 492, 1932.
7. Waerhaug, J.: The Gingival Pocket, Anatomy, Pathology, Deepening and Elimination, *Odontologisk Tidsskrift* **60**: (Supp. 1) 1952.
8. *Idem:* Tissue Reaction Around Artificial Crowns, *J. Periodont.* **24**: 172, 1953.
9. *Idem:* Microscopic Demonstration of Tissue Reaction Incident to Removal of Subgingival Calculus, *J. Periodont.* **26**: 26, 1955.
10. *Idem:* Enamel Cuticle, *J. D. Res.* **35**: 313, 1956.
11. *Idem:* Control of the Endpoint of Decalcification by Fluoroscopy, *Stain Technol.* **29**: 213, 1954.
12. Zander, H. A.: A Method for Studying "The Epithelial Attachment," *J. D. Res.* **35**: 308, 1956.

MESIODISTAL CROWN DIAMETERS OF THE DECIDUOUS AND PERMANENT TEETH IN INDIVIDUALS

COENRAAD F. A. MOORREES, SELMA Ø. THOMSEN, ELLI JENSEN, AND PETER KAI-JEN YEN

The Forsyth Dental Infirmary for Children, Boston, Mass.

SINCE the crowding and spacing of teeth depend in large measure on the relationship between the size of the bony dental arch and the size of the teeth,[5] the importance of mesiodistal crown diameter as one of the two factors in the equation is clear. The relationship between the mesiodistal crown diameters of the deciduous and permanent teeth likewise plays an important role in the development of occlusion of the permanent dentition. Little information is available concerning this phase of dental development because the longitudinal data needed for its study are difficult to obtain.

MATERIAL

The material for the present study consisted of plaster casts of the dentitions of 184 North American children of European stock (91 males and 93 females), studied longitudinally from 3 to 18 years of age. Most of these casts (132) were collected as part of the study of "Maternal and Child Health" directed by Dr. Harold C. Stuart of the School of Public Health, Harvard University.[8] The others (52) were gathered by Dr. Richard H. Stucklen in the Bayard Public School in Wilmington, Delaware.

Impressions of the deciduous dentitions were made with modeling compound and those of the permanent dentitions with a hydrocolloid material.

METHODS

The mesiodistal crown diameter of a tooth was obtained by measuring the greatest distance between the contact points on its approximal surfaces, using a sliding caliper held parallel both to the occlusal and vestibular surfaces. This technic could be employed only when the teeth were in a "normal" position in the dental arch. Otherwise, the mesiodistal crown diameter was obtained by measuring between the points where contact with the neighboring teeth "normally" should occur. Following the method described, the "mean length," as defined by Remane,[6] was measured.* However, in this report the term "mesiodistal crown diameter" has been used since no other term can satisfactorily be substituted for it.

Presented in part, at the Thirty-third General Meeting of the International Association for Dental Research in Chicago, Ill., March 20, 1955 (*J. D. Res.* 34: 714, 1955).

This study was supported in part by a grant from the Charles H. Hood Dairy Foundation of Boston, Mass.

Received for publication July 5, 1955, revised by authors July 3, 1956.

*Mittellänge. Grösster Abstand der mesialen Fläche von der distalen, in der mittleren Längsebene parallel zur Längsachse.

The errors of measurement by different investigators, both of mesiodistal crown diameters of individual permanent teeth and of the combined mesiodistal crown diameters of a series of such teeth (Pm_2 – Pm_2), were calculated from a number of double determinations and expressed as standard deviations of the differences. For single permanent mandibular teeth of different tooth classes the error of measurement was 0.09 mm. (n = 360) and for the combined mesiodistal crown diameters of ten permanent mandibular teeth (Pm_2 – Pm_2) it was 0.41 mm. (n = 30).

For each tooth class statistical analysis of the data on this crown dimension is based on average diameter of the teeth on the right and left side of each dental cast. When, occasionally, the antimere of a tooth is missing, the mesiodistal crown diameter of the one tooth present is used. For these reasons, the "n" values in Tables I and II do not represent the total number of teeth measured, but refer to the number of individuals studied.

TABLE I
MESIODISTAL CROWN DIAMETERS OF DECIDUOUS TEETH OF NORTH AMERICAN WHITE CHILDREN (MM.)

		M	S.E.M	S.D.	RANGE	n	C.R.	p
				Maxilla				
di_1	♂	6.55	0.05	0.36	5.8- 7.2	64	1.56	0.1 <p<0.15
	♀	6.44	0.05	0.43	5.4- 7.5	69		
di_2	♂	5.32	0.05	0.39	4.5- 6.6	64	1.41	0.15 <p<0.16
	♀	5.23	0.04	0.33	4.5- 6.2	69		
dc	♂	6.88	0.04	0.36	6.1- 7.9	65	3.68	p<0.001
	♀	6.67	0.04	0.35	5.9- 7.6	69		
dm_1	♂	7.12	0.05	0.38	6.3- 8.3	64	2.66	0.001<p<0.01
	♀	6.95	0.04	0.36	6.3- 7.9	68		
dm_2	♂	9.08	0.06	0.46	8.0-10.4	63	2.61	0.001<p<0.01
	♀	8.84	0.07	0.55	7.5-10.0	68		
				Mandible				
di_1	♂	4.08	0.04	0.30	3.0- 4.7	64	1.75	0.05 <p<0.1
	♀	3.98	0.04	0.30	3.2- 4.7	68		
di_2	♂	4.74	0.04	0.35	4.1- 6.0	65	1.72	0.05 <p<0.1
	♀	4.63	0.05	0.39	3.9- 5.7	69		
dc	♂	5.92	0.04	0.32	5.1- 6.7	65	3.18	0.001<p<0.01
	♀	5.74	0.04	0.35	5.0- 6.6	68		
dm_1	♂	7.80	0.05	0.42	7.0- 8.9	65	2.34	0.01 <p<0.05
	♀	7.65	0.04	0.35	6.7- 8.5	69		
dm_2	♂	9.83	0.07	0.52	8.5-11.0	63	2.07	0.01 <p<0.05
	♀	9.64	0.06	0.49	8.6-10.9	69		

This procedure was used to obtain the correct standard errors of the means, since the magnitude of a standard error is inversely proportional to the number of observations, i.e., the larger this number, the smaller the error. By counting the mesiodistal crown diameters for both the right and left teeth of each tooth class separately, the sample no longer consists of completely independent observations, because a tooth and its antimere are influenced by

TABLE II
MESIODISTAL CROWN DIAMETERS OF PERMANENT TEETH OF NORTH AMERICAN WHITE CHILDREN (MM.)

		M	$S.E._M$	S.D.	RANGE	n	C.R.	p
				Maxilla				
I_1	♂	8.78	0.05	0.46	7.9-10.0	87	4.87	$p<0.001$
	♀	8.40	0.06	0.53	7.1- 9.8	87		
I_2	♂	6.64	0.07	0.63	4.5- 8.2	84	1.72	$0.05<p<0.1$
	♀	6.47	0.07	0.62	4.5- 8.5	86		
C	♂	7.95	0.05	0.42	6.6- 9.0	87	6.56	$p<0.001$
	♀	7.53	0.04	0.37	6.9- 8.5	85		
Pm_1	♂	7.01	0.04	0.38	6.1- 8.2	87	2.50	$0.01<p<0.05$
	♀	6.85	0.05	0.42	5.8- 7.8	84		
Pm_2	♂	6.82	0.04	0.37	5.9- 7.6	86	3.13	$0.001<p<0.01$
	♀	6.62	0.05	0.43	5.2- 7.8	81		
M_1	♂	10.81	0.06	0.56	9.9-12.4	83	3.41	$p<0.001$
	♀	10.52	0.06	0.51	9.4-11.9	85		
M_2	♂	10.35	0.08	0.63	8.5-11.7	65	5.09	$p<0.001$
	♀	9.81	0.07	0.49	8.3-11.8	50		
				Mandible				
I_1	♂	5.42	0.03	0.31	4.5- 6.1	85	3.40	$p<0.001$
	♀	5.25	0.04	0.36	4.3- 6.1	87		
I_2	♂	5.95	0 04	0.38	5.2- 6.9	85	2.98	$0.001<p<0.01$
	♀	5.78	0.04	0.38	4.7- 6.8	87		
C	♂	6.96	0.04	0.36	6.2- 8.1	84	8.60	$p<0.001$
	♀	6.47	0.04	0.32	5.8- 7.4	87		
Pm_1	♂	7.07	0.04	0.35	6.4- 8.1	85	3.51	$p<0.001$
	♀	6.87	0.04	0.38	5.9- 7.9	87		
Pm_2	♂	7.29	0.06	0.52	6.3- 9.6	82	3.75	$p<0.001$
	♀	7.02	0.04	0.40	6.2- 7.9	83		
M_1	♂	11.18	0.05	0.47	10.0-12.7	76	5.64	$p<0.001$
	♀	10.74	0.06	0.56	9.4-12.2	84		
M_2	♂	10.76	0.10	0.71	9.3-12.5	53	3.28	$p<0.001$
	♀	10.34	0.08	0.62	9.2-11.7	53		

similar, although not necessarily identical, genetic and environmental factors. For this reason one may be counting the end result of the same factor complex twice in a majority of instances when basing the calculations on the number of individual teeth. The smaller standard error thus obtained may lead to an overestimate of the true level of statistical significance when analyzing the difference between the two means.

FINDINGS

The data on mesiodistal crown diameters of the deciduous and permanent maxillary and mandibular teeth of the North American white children studied are summarized in Tables I and II.

The tooth crowns of the males are seen to be invariably broader than those of the females. This sex difference is larger for the permanent than for the deciduous teeth, as indicated by the critical ratios, and it is more pronounced for the canines than for any other teeth in both dentitions.

Lundström's[2] and Seipel's[7] findings on mesiodistal crown diameters of Swedes are the only other data that furnish sufficient statistics for comparative studies dealing with populations of white stock. Seipel made all of his measurements of the deciduous and permanent teeth directly, without resorting to impressions and casts, while Lundström obtained his material by measuring the permanent incisors and canines directly in the mouth and the premolars and molars on dental casts. According to Lundström,[1] the differences obtained by the direct and indirect technics are very small.

The mean mesiodistal crown diameters of the deciduous teeth are smaller for the North American children studied here than for the Swedish children measured by Seipel except in the case of the deciduous maxillary second molars. For both sexes these differences are statistically significant, with the exception of those for the deciduous maxillary central incisors and the maxillary and mandibular second molars.

In general, judging from the mean mesiodistal crown diameter obtained, the permanent teeth of the North American children are also smaller, although not always significantly smaller than the teeth in the two samples of Swedish children. In the few instances where the opposite relationship seems to exist, the differences found have no statistical significance.

When comparing the mean mesiodistal crown diameters for the two different samples of Swedish children, statistically significant differences are found for the permanent maxillary and mandibular incisors and canines of the boys and the permanent maxillary and mandibular canines of the girls. The children studied by Seipel have a larger mesiodistal diameter of the tooth crowns than those studied by Lundström, except in the case of the mandibular lateral incisor of the boys (which is markedly smaller in Seipel's series than in that of Lundström), and possibly also in three other instances (although for these teeth the differences do not exceed the error of measurement).

The value of odontometry in population studies is not sufficiently established[3] but the comparisons between the two groups of Swedish children

indicate that it may be necessary to measure teeth in more than one sample of a population before making conclusive statements concerning the odontometric findings and the racial origin of a population.

Inasmuch as the main purpose of the present study is of an orthodontic nature dealing with the space occupied by the teeth in the dental arch, the findings on mesiodistal crown diameters of single teeth have been combined to obtain the mesiodistal crown diameters of certain groups of teeth.[4]

The relationships between the mesiodistal crown diameters of the deciduous and permanent incisors and canines, as well as those between the deciduous molars and their permanent successors, the premolars, have been determined also by expressing the combined crown diameters of the deciduous teeth as a percentage of that of the permanent teeth.

These data are consistent with the usual finding according to which the permanent incisors and canines are larger, but the premolars smaller than their deciduous predecessors. However, a marked range of individual departures from the mean values can be noted from the tabulations. In some instances, the exfoliation of the deciduous molars provides a considerable amount of space in excess of that needed for the emerging premolars, while in other instances, the difference in space requirements is negligible.

A large surplus of space may occur in the maxilla, as well as in the mandible. That this happens more often in the mandible than in the maxilla is made evident by comparing the mean indices expressing the relationship between the mesiodistal crown diameters of the deciduous mandibular molars and the successional premolars in the two jaws.[4]

The combined mesiodistal crown diameters of the maxillary and mandibular deciduous teeth ($dm_2 - dm_2$) and of their permanent successors ($Pm_2 - Pm_2$) are shown in Table III. The mean value of these combined crown diameters of the maxillary permanent teeth is 5.22 mm. larger than that of their deciduous predecessors in the males, and 3.59 mm. larger in the females. In the mandible, the permanent teeth of the males are only 0.77 mm. larger than the deciduous teeth. In the females, on the other hand, the difference between the means is even less, or only 0.17 mm.

The critical ratios of the differences between the mean combined mesiodistal crown diameters in the males and females are again higher for the permanent than for the deciduous dentition, and these differences are statistically significant in all instances (Table III).

The relationship between the mesiodistal crown diameters of the deciduous teeth and their permanent successors has been computed from the formula:

$$\frac{\text{Sum of mesiodistal crown diameters of deciduous teeth } (dm_2 - dm_2)}{\text{Sum of mesiodistal crown diameters of permanent teeth } (Pm_2 - Pm_2)} \times 100.$$

The mean figure expressing the sum of the mesiodistal crown diameters of the deciduous teeth in terms of a percentage of that of the corresponding permanent teeth is 93 for the maxillary dentition of the males and 95 for the

females; in the mandibular dentition the corresponding percentages are 98 and 100, respectively. These findings support the conclusions previously made from the absolute measurements (Table III).

TABLE III
COMBINED MESIODISTAL CROWN DIAMETERS OF THE DECIDUOUS TEETH AND THEIR PERMANENT SUCCESSORS (MM.)

		M	$S.E._M$	S.D.	RANGE	n	C.R.	p
Deciduous teeth ($dm_2 - dm_2$)								
Maxilla	♂	69.69	0.37	2.89	63.8-76.9	60	3.17	$0.001<p<0.01$
	♀	68.04	0.38	3.04	62.5-76.1	65		
Mandible	♂	64.52	0.37	2.86	59.2-71.9	59	2.57	$p<0.01$
	♀	63.21	0.35	2.83	56.4-67.4	65		
Permanent teeth ($Pm_2 - Pm_2$)								
Maxilla	♂	74.91	0.44	3.38	68.4-84.2	57	5.27	$p<0.001$
	♀	71.63	0.44	3.36	62.4-79.4	57		
Mandible	♂	65.29	0.39	2.93	57.4-72.7	57	4.51	$p<0.001$
	♀	63.04	0.39	3.11	57.2-70.4	62		

It may be pointed out, however, that a considerable range of variation exists between the combined mesiodistal crown diameters of the deciduous and permanent teeth ($Pm_2 - Pm_2$) in the individual children studied.

One advantage of a longitudinal study is the possibility of calculating the correlation coefficient or the degree of association between the mesiodistal crown diameters of the deciduous and permanent teeth in the same subject. Although differing significantly from zero, except for the maxillary lateral incisors in males, the degree of association between the mesiodistal crown diameters of the deciduous and permanent teeth of each tooth class was found to be low to

TABLE IV
CORRELATION COEFFICIENTS FOR MESIODISTAL CROWN DIAMETERS OF CORRESPONDING SINGLE AND COMBINED DECIDUOUS AND PERMANENT TEETH

	MALES			FEMALES		
	r	$S.E._r$	n	r	$S.E._r$	n
Maxilla						
$di_1 : I_1$	+0.56	±0.09	64	+0.65	±0.07	66
$di_2 : I_2$	+0.23*	±0.12	61	+0.41	±0.10	65
dc : C	+0.26	±0.12	64	+0.34	±0.11	64
$dm_1 : Pm_1$	+0.27	±0.12	63	+0.34	±0.11	62
$dm_2 : Pm_2$	+0.34	±0.11	62	+0.45	±0.10	59
$dm_2 - dm_2 : Pm_2 - Pm_2$	+0.54	±0.10	56	+0.69	±0.07	57
Mandible						
$di_1 : I_1$	+0.43	±0.10	62	+0.37	±0.11	65
$di_2 : I_2$	+0.35	±0.11	63	+0.39	±0.10	66
dc : C	+0.28	±0.12	63	+0.32	±0.11	65
$dm_1 : Pm_1$	+0.41	±0.11	63	+0.53	±0.09	64
$dm_2 : Pm_2$	+0.37	±0.11	58	+0.42	±0.10	64
$dm_2 - dm_2 : Pm_2 - Pm_2$	+0.59	±0.09	55	+0.49	±0.10	61

*The correlation coefficient does not differ significantly from zero.

medium with coefficients ranging from +0.23 to +0.65 (Table IV). Thus, in general, deciduous teeth with a small mesiodistal crown diameter are succeeded by permanent teeth which are likewise small in their mesiodistal dimension, while broad deciduous teeth are likely to be followed by broad permanent ones. Yet,

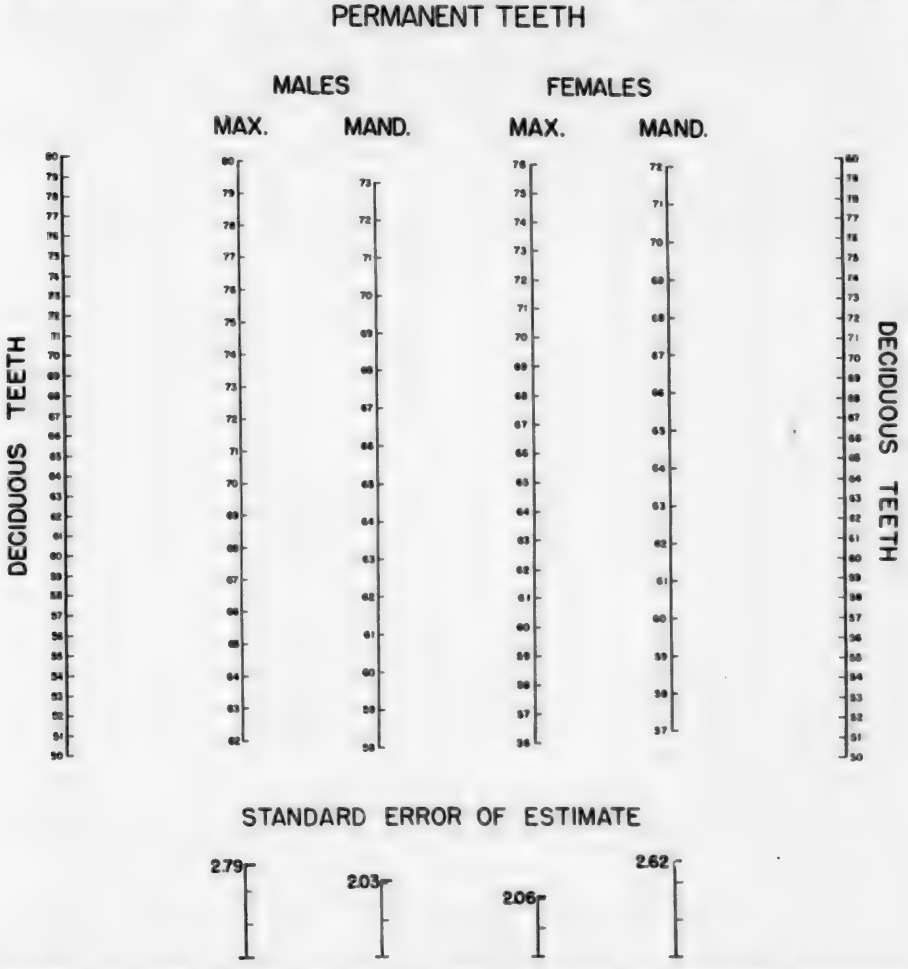


Fig. 1.—Nomograph for estimating the sum of the mesiodistal crown diameters of permanent teeth ($Pm_2 - Pm_2$) from that of the deciduous teeth ($dm_2 - dm_2$).

the relatively low numerical values of the correlation coefficients indicate that in individual instances a great deal of variation can be expected in the size of a permanent tooth replacing a deciduous tooth of a given mesiodistal crown diameter.

The highest correlation coefficient was found for the mesiodistal crown diameters of the deciduous and permanent maxillary central incisors ($r = +0.56$

for males and +0.65 for females). It is obvious, then, that no accurate prediction of the mesiodistal crown diameter of the permanent tooth can be made even in this particular instance, on the basis of a measurement of its deciduous predecessor.

The correlation coefficients for the combined mesiodistal crown diameters of the incisors and canines in the deciduous and permanent dentitions, as well as those for the deciduous molars and premolars, are also from low to medium in magnitude for both males and females, the range of r being from +0.38 to +0.60.[4] A negligibly higher degree of association is found between the sum of the mesiodistal crown diameters of the deciduous and permanent teeth (Pm_2 – Pm_2), as shown in Table IV.

Regression equations were calculated from the data on the combined crown diameters making it possible to draw a nomograph for assessing the sum of the mesiodistal crown diameters of the permanent teeth (Pm_2 – Pm_2) when that of the deciduous teeth is known (Fig. 1). The errors of estimate in such determinations are rather large, varying between 2.03 and 2.79 mm. This means that at the 95 per cent level of confidence the true size of the permanent teeth (Pm_2 – Pm_2) in an individual is within a range of plus and minus two times the error of estimate from the expected value.

The nomograph is, therefore, only of academic value for predictive purposes, and in actual practice it is preferable to determine the size of the permanent teeth from radiographs taken after the crowns of the teeth are sufficiently calcified.

SUMMARY AND CONCLUSIONS

As one aspect of a longitudinal study, mesiodistal crown diameters were obtained by measurements of plaster casts of the deciduous and permanent dentitions of 184 North American children of European ethnic origin. The statistical analysis of the data for single teeth and for groups of teeth is presented in tabular form.

The relationship between the mesiodistal crown diameters of the deciduous and permanent teeth has been described by means of indices and correlation coefficients.

Despite the fact that, on the average, the permanent teeth exceed the deciduous teeth in mesiodistal crown diameter, the former can be either smaller or larger than their deciduous predecessors. The combined mesiodistal crown diameters also may be the same in the two dentitions, so that the permanent teeth (Pm_2 – Pm_2) occupy the same amount of space in the dental arch as did the deciduous teeth.

The authors are indebted to Drs. Harold C. Stuart and Richard H. Stucklen for the use of their material. Dr. Veikko O. Hurme, Director of Clinical Research of the Forsyth Dental Infirmary for Children, contributed greatly to this paper by a critical review of the manuscript. The authors also want to thank Miss Maria Krywoshlikow of the Orthodontic Department, Forsyth Dental Infirmary, for making statistical comparisons of data pertaining to the teeth of Swedes.

REFERENCES

1. Lundström, A.: Intermaxillära tandbreddsförhållanden och tandställningen, *Svensk Tandl.-Tidskrift* **36**: 575, 1943.
2. Lundström, A.: Förtidiga mjölktandsförluster och tandställningen, *Svensk Tandl.-Tidskrift* **37**: 698, 1944.
3. Moorrees, C. F. A.: The Aleut Dentition, Cambridge, Mass., 1957, Harvard University Press. To be published.
4. Moorrees, C. F. A., et al.: The Dentition of the Growing Child, Manuscript, 1957.
5. Moorrees, C. F. A., and Reed, R. B.: Biometrics of Crowding and Spacing of the Teeth in the Mandible, *Am. J. Phys. Anthropol.* n.s. **12**: 77, 1954.
6. Remane, A.: Zur Messtechnik der Primatenzähne, in E. Abderhalden; Handbuch der biologischen Arbeitsmethoden 7: part 1, Berlin-Wien, 1930, Urban & Schwarzenberg, p. 609.
7. Seipel, C. M.: Variation of Tooth Position, *Svensk Tandl.-Tidskrift* **39**: Suppl., 1946.
8. Stuart, H. C., and Staff: Studies From the Center for Research in Child Health and Development, School of Public Health, Harvard University, 1. The Center, the Group Under Observation, Sources of Information, and Studies in Progress, Monographs of the Soc. for Research in Child Develop. 4, Serial No. 20, number 1, 1939.

HISTOLOGIC CHANGES IN THE HAMSTER CHEEK POUCH DURING EARLY HYDROCARBON CARCINOGENESIS

JOHN J. SALLEY*

Division of Dental Research and Department of Pathology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

IN A previous investigation,[6] the cheek pouch of the Syrian hamster was subjected to treatment by 3 carcinogenic hydrocarbons: 9,10-dimethyl-1,2-benzanthracene, 20-methylcholanthrene and 3,4-benzpyrene. Neoplasms ranging from benign papilloma to squamous-cell carcinoma with regional lymph node metastasis were produced. The most potent carcinogen for oral tumor induction was found to be 9,10-dimethyl-1,2-benzanthracene in an acetone solution. Later work[7] has shown that the average latent period for tumor appearance with this compound could be significantly shortened from 6 to 7 weeks when acetone was used, to 4½ weeks with mineral oil as a solvent.

Although different histologic grades of preneoplasia and neoplasia were described in the earlier report, many animals exhibited changes ranging from hyperplasia to frankly malignant tumors in the same pouch. This may possibly be explained on the basis of different areas of the pouch responding to carcinogenic treatment at different times. It was felt that a study whereby progressive changes in oral epithelium could be observed before neoplastic transformation would be useful.

Investigations of this type have been reported previously for epidermal tissue treated with methylcholanthrene[1, 4] and benzpyrene.[2] Generally, these reports concur in their descriptions of the histogenesis of experimental skin carcinoma. The initial reaction after treatment with the carcinogen was inflammation. This was followed by a period of degeneration which, in turn, was replaced by a stage where epithelial hyperplasia predominated. The first evidence of neoplastic change was the appearance of epithelial papillomata. These benign tumors then underwent malignant transformation to squamous-cell carcinoma. Orr[5] has described essentially the same findings in subcutaneous tissue implanted with wax pellets containing methylcholanthrene, 3,4-benzpyrene and 1,2,5,6-dibenzanthracene; however, in this latter instance the tumor induced was sarcomatous. Levy, Gorlin, and Gottsegen[3] made a single application of 9,10-dimethyl-1,2-benzanthracene to the skin and mucous membrane of the lips of Swiss albino mice. Animals were sacrificed daily up to 35 days. Their findings on skin were compatible with those described above. In the mucous membrane they reported increased surface keratinization; slight hyperplasia of the

This study was supported by a grant-in-aid from the National Institutes of Health, Bethesda, Md.

Received for publication Oct. 17, 1955.

*Present address: Department of Pathology, Medical College of Virginia, Richmond 19, Va.

epithelial cells, particularly in the stratum germinativum; hydropic changes in the stratum spinosum and stratum granulosum; and thickening and edema of the lamina propria. No neoplastic changes were described.

A point of interest in regard to studies of epithelial carcinogenesis on skin is the importance in the carcinogenic process attributed to the hair follicles and sebaceous glands. Several authors have stressed the importance of the hair follicle in skin tumor induction.[1, 4, 8] The basis for this opinion was the observation that these structures degenerated in the early stages of the process, only to regenerate and become hyperplastic, forming the centers of the induced tumors which followed. Simpson and Cramer,[9] in their report dealing with early changes induced in skin by methylcholanthrene as studied by fluorescence microscopy, pointed out the importance of the sebaceous glands which took up the hydrocarbon 2 to 3 minutes after the first application. There was then degeneration of the gland with the sebum containing the carcinogen excreted into the hair follicles, and thence onto the surface.

It has been indicated previously[6] that there are no accessory or other structures in the hamster cheek pouch as examined by serial histologic sections which could possibly play a similar role in the carcinogenic process in this structure. The following study was therefore instituted to ascertain the histologic alterations produced in the cheek pouch wall at successive stages during the process of tumor induction by 9,10-dimethyl-1,2-benzanthracene; and to compare these findings with those that have been previously described for skin.

EXPERIMENTAL PROCEDURE

A group of 24 hamsters, 3 months of age, had both cheek pouches painted with 9,10-dimethyl-1,2-benzanthracene in a 0.5 per cent concentration with mineral oil, U.S.P. heavy grade, as a solvent. The pouches were treated 3 times per week, and the painting technique was identical with that which has been described earlier.[6] Twenty-four hours after each application of the carcinogenic solution, one animal was sacrificed by chloroform inhalation. The treated pouches were inverted and removed in toto and fixed in a solution of 1 per cent trichloroacetic acid in 80 per cent ethyl alcohol. After fixation and dehydration, a piece of tissue 1 cm. square including both medial and lateral walls was dissected out at about the middle depth of the pouch and was embedded in paraffin. Sections were cut at 7 microns and stained with hematoxylin and eosin.

A total of 16 applications of the hydrocarbon was made, and with the procedure just described it was possible to study any histologic tissue change which became apparent with each treatment. The experimental period was 9 weeks, although painting was terminated after the first appearance of tumors (5 weeks).

OBSERVATIONS AND RESULTS

Grossly, observations of inflammation and edema could be made after the initial and second to third applications. After 8 or 9 treatments, there were raised, whitish lesions on the painted epithelium not unlike leukoplakia in human oral mucosa. The first evidence of tumor was seen in the hamster which had been treated with 15 applications of the hydrocarbon solution. This consisted of a small, raised nodule measuring approximately 0.5 mm. in diameter.

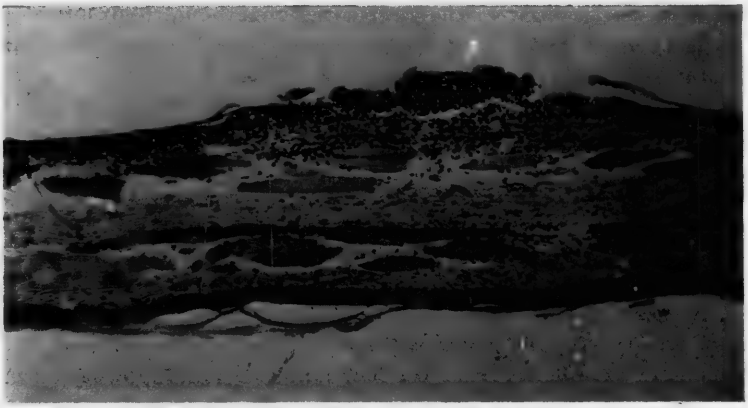


Fig. 1.—Pouch wall 24 hours after 1 application of 9,10-dimethyl-1,2-benzanthracene. Note inflammatory reaction which has resulted in ulceration of epithelium. (Hematoxylin and eosin; ×100.)

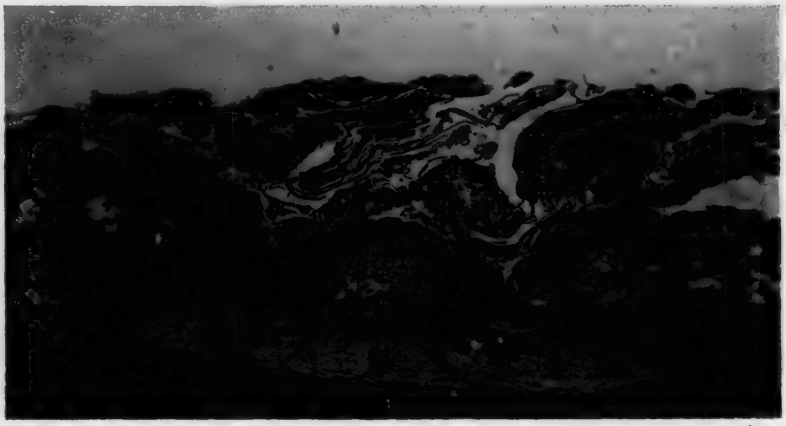


Fig. 2.—Pouch wall after 3 applications of the carcinogen. This stage was characterized by degeneration and necrosis with some sloughing. There is thickening in all layers. (Hematoxylin and eosin; ×100.)

Fig. 3.—The appearance of the pouch wall at the fourth and fifth applications. This stage was designated as regenerative. (Hematoxylin and eosin; ×100.)

From this point onward all pouches exhibited papillomatous growths and several were allowed to grow until they reached a size in excess of 1 cm. in diameter.

Histologically, changes were apparent in pouches treated with only one application of 9,10-dimethyl-1,2-benzanthracene. These were characterized by inflammation with the presence of inflammatory cells in the epithelium as well as the submucosal connective tissue and striated muscle layers. Fig. 1 is a photomicrograph of a pouch 24 hours after the first painting. In this section a focus of inflammation can be seen wherein the surface epithelium has been partially destroyed leaving an ulcerated area. There are large numbers of inflammatory cells, primarily mononuclear leukocytes although some polymorphonuclear leukocytes are also present, both above and below the epithelium. The epithelium as a whole appears to be thinned out and the keratin layer is reduced in thickness. On the opposite wall of the pouch there is no inflammation and it presents a relatively normal appearance. It would appear that this area represents the lateral wall and did not receive as great a concentration of the hydrocarbon. However, there is a small aggregation of inflammatory cells in the muscle layer beneath it, although not as extensive as the medial wall. Essentially the same findings were noted in the pouches which had received 2 treatments.

Fig. 2 shows a pouch which was subjected to 3 treatments of the carcinogen. Here the inflammatory reaction appears to have subsided in intensity, especially in the submucosal connective tissue and the muscular layer. There is still some inflammation present in the epithelium, but the most prominent feature in this tissue is degeneration and necrosis. Here one sees epithelial cells with pyknotic nuclei which are separated by what appears to be precipitated fibrin and cellular debris. Some chronic inflammatory cells are present. The most striking change is the marked thickening of this layer caused by scattered masses of necrotic tissue and shreads of keratin embedded on and between the degenerating epithelial cells. The remainder of the pouch wall is free of these alterations, although the dense connective tissue of the submucosa may be somewhat increased in thickness; and there are some scattered mononuclear cells between the muscle fibers. From the fourth through the fifth applications there was an apparent regeneration of the epithelium. No evidence of inflammation or degeneration was present. At these stages the epithelium appeared to be reduced in thickness to no more than 2 cell layers. Keratinization on the surface was negligible, and there were no remarkable changes in the other three layers. This stage is illustrated in Fig. 3.

Commencing with 6 applications of the carcinogen through the appearance of neoplasia at 15 applications, there was a period of hyperplasia especially in the epithelial layer. An example of this phase of oral carcinogenesis is shown in Fig. 4. Here, the epithelium has become thickened from the normal of 2 to 4 cell layers to 6 and 8 layers. There is also evidence of increased keratin formation on the surface. The cells of the stratum germinativum which normally are arranged in straight line on the connective tissue now appear to have a peg-like morphology, indicating some downward growth of the epithelium. Increased activity of these cells is also emphasized by their hyperchromatism in

relation to normal basal cells. Proliferative activity is present in the submucosal connective tissue, where one can see a predominance of cellular elements in one area as contrasted with the adjacent portion where more mature connective tissue characterized by many fibers is present. The increased staining quality of the latter is also apparent. In addition, the entire submucosal connective tissue appears to be thickened with increased vascularity; and there is evidence of proliferative activity in the loose areolar connective tissue making up the outer layer of the pouch wall. This hyperplastic reaction gradually increased in scope until there was definite evidence of neoplastic change.

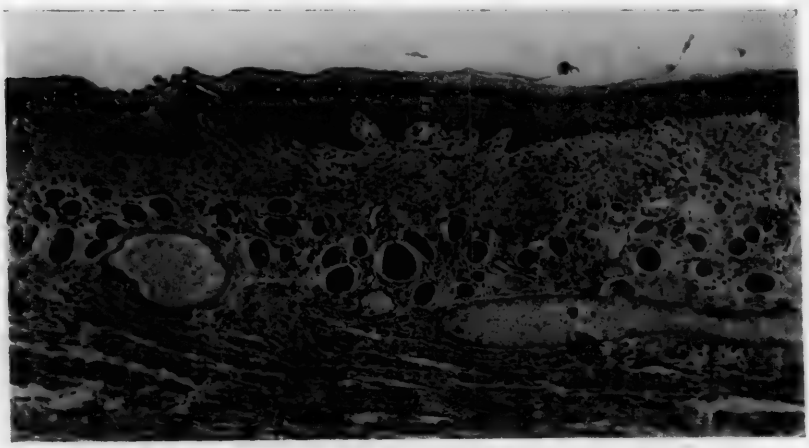


Fig. 4.—The pouch at 8 applications of 9,10-dimethyl-1,2-benzanthracene. There is increased activity in all layers of the pouch wall. (Hematoxylin and eosin; ×100.)

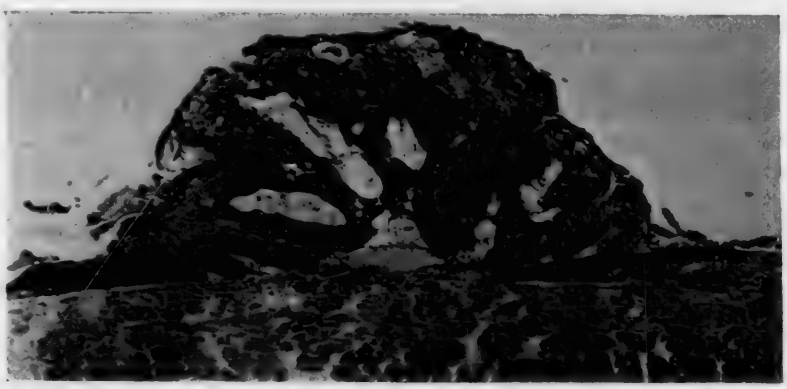


Fig. 5.—Benign papilloma first seen at 15 applications of the carcinogen. (Hematoxylin and eosin; ×60.)

At 15 applications and thereafter, papillomatous tumors were present in the pouches. Generally, these tumors did not extend into the connective tissue, but were outgrowths of epithelial cells from the surface. They showed some keratotic crypts with hyperkeratosis and some parakeratosis. Fig. 5 is an example of the neoplasms seen at this stage. This tumor is benign; although

there is some cellular atypicality with the presence of prominent nucleoli, hyperchromatism, and a change in cell size. In addition, there is acanthosis present, as well as evidence of increased vascular supply at the base of the growth. Fig. 6 is a section of a tumor from a pouch removed 7½ weeks subsequent to the beginning of the study. Here the diagnosis of malignancy is equivocal since in some areas there are atypical cells showing hyperchromatism and loss of stratification with some abnormal mitotic figures, while other fields are characterized more by hyperplastic-appearing cells. In some areas there appears to

Fig. 6.—A section from the base of a tumor after 7½ weeks of the experimental period. (Hematoxylin and eosin; ×60.)

be loss of continuity of the basement membrane associated with many small, acanthotic epithelial cells. There are numerous epithelial pearls present and there is hyperkeratosis and parakeratosis on the surface. The connective tissue supporting the tumor consists of loose, irregularly arranged fibers surrounding many large sinusoid-like structures which contain blood elements. Throughout the tumor there are small, scattered foci of chronic inflammatory cells.

Fig. 7 illustrates a malignant carcinoma of the squamous-cell type removed from a pouch after 9 weeks. This tumor is characterized by a marked invasion of the tissue underlying the epithelium by nests and sheets of malignant epithelial cells. There is hyperkeratosis and parakeratosis, the presence of keratotic crypts and large vacuolated, hyperchromatic cells with nuclear atypicality, prominent nucleoli and many atypical mitotic figures. The entire mass consists of infiltrating squamous cells on a network of loosely arranged connective

tissue fibers. Regional lymph nodes were removed and examined during the latter part of the experiment, but metastasis, which was described in the previous investigation,[6] was not noted in this study probably due to the shorter experimental period.

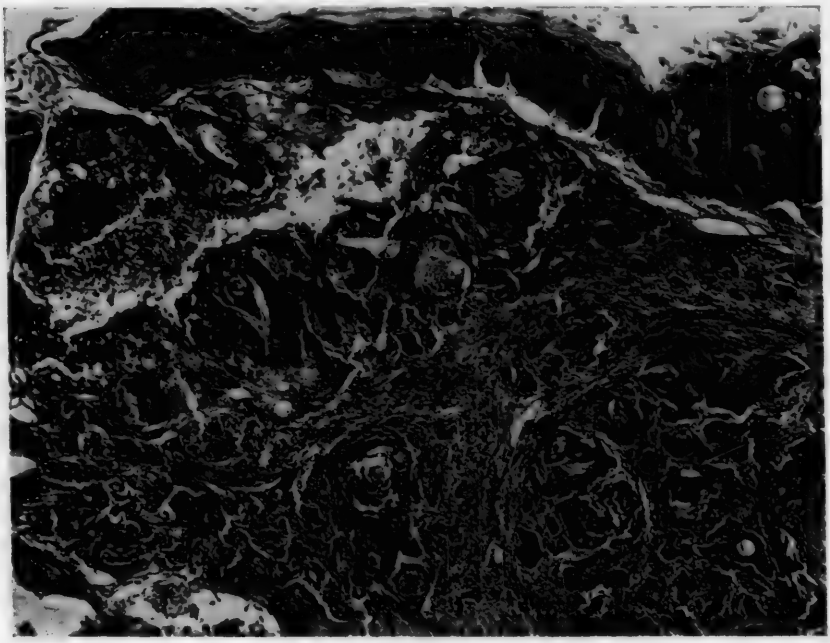


Fig. 7.—Squamous-cell carcinoma removed from a pouch after 9 weeks. (Hematoxylin and eosin; ×60.)

DISCUSSION

The results just presented indicate that oral epithelium subjected to hydrocarbon carcinogenesis passes through similar stages histologically as have been described for skin treated with chemical carcinogens. The reaction of the oral mucosa may be divided into 4 stages before neoplastic transformation: inflammation, degeneration characterized by necrosis, regeneration, and hyperplasia. It is interesting to note that when inflammation subsided after the first few treatments, it was never observed again even though the tissue was continually being insulted with what appeared in the initial stages to be a fairly strong irritant. The reason for this finding is not known unless the exposed cells became acclimated to the irritant in the relatively short period of one week. If this be true, the periods of regeneration and particularly hyperplasia should naturally follow since continued exposure to irritation would have a hyperplastic response as its usual sequel.

It has been stated that in some species of animals, experimentally induced benign tumors of the skin will regress if treatment is terminated.[10] However, in this study treatment was discontinued after the first appearance of benign neoplasms without any sign of regression. Fig. 7 shows a squamous-cell carcinoma which arose from a benign epithelial papilloma induced after 15 treat-

ments, but which did not have malignant characteristics until approximately 4 weeks later. Even though regression of skin tumors has been described, a major factor in this phenomenon appears to be the species of animal (the rabbit exhibiting the greatest susceptibility to regression).[10] It may be indicated here that extrapolation of data relative to experimental tumor induction in one species should be made to other species and other anatomical sites in the same species with many reservations.

The last statement may also apply to the importance of the role of accessory structures in carcinogenesis. Hair follicles and sebaceous glands, especially the former, may well be important requisites in the induction of skin tumors, but their absence or the absence of other related structures in the pouch mucosa (e.g., mucous glands) seemed to exert little influence on the response of treated tissue. Similarly, the induction of connective tissue malignancies by hydrocarbon implantation technics is successful, also without the presence of accessory structures or glands. Therefore, on this basis it is difficult to make a comparison between skin and mucous membrane relative to their response to carcinogenic treatment since these two tissues, although somewhat alike histologically, have different environment, and physiological activity, and so forth.

SUMMARY

A study has been described wherein the early changes associated with 9,10-dimethyl-1,2-benzanthracene carcinogenesis in the hamster cheek pouch have been observed by conventional histologic methods. The pouch wall appeared to pass through 4 distinct histologic gradations before the appearance of definite neoplasms. These were inflammation, degeneration, regeneration, and hyperplasia. Descriptions and accompanying illustrations of each of the 4 stages plus the type of tumor which resulted have been included. A brief discussion comparing skin and oral mucosa and their reaction to experimental tumor induction has also been presented.

REFERENCES

1. Cramer, W., and Stowell, R. E.: The Early Changes of Carcinogenesis by 20-Methylcholanthrene in the Skin of the Mouse. II. Microscopic Tissue Changes, *J. Nat. Cancer Inst.* **2**: 379, 1942.
2. Glucksman, A.: The Histogenesis of Benzpyrene-induced Epidermal Tumors in the Mouse, *Cancer Res.* **5**: 385, 1945.
3. Levy, B. M., Gorlin, R., and Gottsegen, R.: A Histologic Study of the Reaction of Skin and Mucous Membrane to a Single Application of the Carcinogen 9,10-Dimethyl-1,2-benzanthracene, *J. D. Res.* **29**: 678, 1950.
4. Liang, H.: Localized Changes in Methylcholanthrene-treated Epidermis, *Cancer Res.* **8**: 211, 1948.
5. Orr, J. W.: An Investigation of the Histological Changes in the Subcutaneous Tissues of Mice During the Induction of Sarcoma by Carcinogenic Hydrocarbons, *J. Path. & Bact.* **49**: 157, 1939.
6. Salley, J. J.: Experimental Carcinogenesis in the Cheek Pouch of the Syrian Hamster, *J. D. Res.* **33**: 253, 1954.
7. Salley, J. J.: The Effect of Mineral Oil as a Solvent for 9,10-Dimethyl-1,2-benzanthracene, *J. D. Res.* **34**: 723, 1955.
8. Silberburg, M., and Silberburg, R.: Hair Growth in the Skin of Guinea Pigs Painted with 20-Methylcholanthrene, *Arch. Path.* **44**: 297, 1947.
9. Simpson, W. L., and Cramer, W.: Fluorescence Studies of Carcinogens in Skin. I. Histological Localization of 20-Methylcholanthrene in Mouse Skin After a Single Application, *Cancer Res.* **3**: 362, 1943.
10. Wolf, George: Chemical Induction of Cancer, Cambridge, Mass., 1952, Harvard Univ. Press.

ACID PRODUCTION FROM DIFFERENT CARBOHYDRATE FOODS IN PLAQUE AND SALIVA

T. G. LUDWIG,* B.D.S., M.S., AND B. G. BIBBY, B.D.S., PH.D., D.M.D.

Eastman Dental Dispensary, Rochester, N. Y.

IN RECENT years, several approaches have been made to the determination of the relative importance of different foods in causing dental decay. These include comparing the persistence and concentration of glucose in the saliva,[1-4] measuring the acid formed in saliva,[5, 6] and estimating decalcification potentials (food retained times acid formed in saliva)[7] after consuming various carbohydrate-containing foods and beverages.

However, what goes on in the saliva is probably of less importance in the causation of decay than what happens to foods in the bacterial accumulations or plaque on the tooth surface. Therefore, it seemed worth while to study acid production in plaques following the eating of various carbohydrate foods and, since no such comparisons have been made, at the same time find out the extent to which acid formation in plaques paralleled that of lactic acid in saliva.

METHODS

To compare acid formation in the dental plaque after eating different foods, it was necessary to select subjects with plaques which produced low pH values. The subjects were chosen on the basis of a preliminary test in which the initial pH of dental plaque scrapings, collected before breakfast from the lingual surfaces of the upper premolar teeth, was determined by use of a one-drop glass electrode and a Beckman potentiometer as described by Fosdick and Campaigne.[8] Then the mouth was rinsed for two minutes with a 20 per cent glucose solution, and, for one-half hour thereafter, pH measurements were made every five minutes in plaque material collected from the same tooth area as before. The subjects producing low pHs were recalled for further tests.

Upon the subsequent mornings of the test, control readings were again taken of the pH of the plaque material before the subjects had eaten. Each person then consumed a liberal portion of the test food, and during the following half hour pH measurements were made of plaque material collected at the same intervals as when the glucose rinse was used. On successive mornings, each person was tested with a fig sandwich cookie, toffee, bread and jam, ice cream, fried potatoes, apple, a cola beverage, grape soda, and unsweetened canned orange juice.

The lactic acid content of the saliva was determined in the way described by Hartles.[9] To establish a base line value for the saliva, the subject fasted

This work was initiated under by a grant provided by the National Institutes of Health and continued with the assistance of the American Bottlers of Carbonated Beverages.

Received for publication Sept. 27, 1955. Revised by the authors March 14, 1956.

*Present address: Dental Clinic, Upper Willis Street, Wellington, New Zealand.

for four hours, after which a 1 ml. sample of resting saliva was collected and immediately analyzed for lactic acid, by use of the method of Barker and Summerson.[10] The subject then ate one of the test foods and at 5-minute intervals from the commencement of eating, 1 ml. samples of his saliva were collected for the next half hour. Suitable dilutions of these samples were made, and the lactic acid content of each was immediately estimated. So that any necessary corrections could be applied, the lactate content of each test food was determined.

It is realized that lactic acid may not be the only acid formed in the saliva, but, since close agreement was obtained with a number of titrations of total salivary acid, it provided an acceptable index of acid production.

RESULTS

When the test subjects used the control 20 per cent glucose rinse, acid was formed rapidly in the plaque material, and within five minutes the test subjects had produced pH readings in the vicinity of 5. After ten minutes, the pH of the plaques commenced to rise, and within twenty to thirty minutes had returned to normal (Table I).

TABLE I
pH OF PLAQUE MATERIAL AFTER TWO-MINUTE RINSING WITH 20 PER CENT GLUCOSE SOLUTION

SUBJECT	BEFORE RINSING	5 MIN.	10 MIN.	15 MIN.	20 MIN.	25 MIN.	30 MIN.
J. L.	7.1	5.0	5.4	5.9	6.3	7.0	7.1
F. T.	7.0	5.2	5.3	5.7	6.6	6.8	6.9
T. B.	7.4	4.9	5.1	5.6	6.4	6.3	6.8
M. M.	6.9	4.8	5.7	5.9	6.2	6.9	7.1
P. T.	7.2	4.8	5.3	5.3	5.9	6.4	6.8

After use of most of the test foods, acid was produced in the plaque in relatively large amounts. Detailed results are not listed for all tests, but results given in Table II are representative of the higher acid-producing foods (fig sandwich cookie, toffee, and bread and jam). When these were eaten, acids were produced in the plaques a little more slowly than after the glucose rinse, but within ten minutes the pH of the plaques had fallen to between 5 and 5.5 for most subjects and remained low for the remainder of the half-hour test period. Only a slight rise in the pH of the plaques occurred in the majority of the subjects after eating the fig sandwich cookie, but after toffee and bread and jam, the return of the pH to normal was quicker.

TABLE II
pH OF PLAQUE MATERIAL AFTER EATING FIG SANDWICH COOKIE

SUBJECT	BEFORE EATING	5 MIN.	10 MIN.	15 MIN.	20 MIN.	25 MIN.	30 MIN.
J. L.	7.1	6.5	5.2	5.7	5.7	5.8	5.8
F. T.	7.2	6.2	5.5	5.6	5.9	6.1	6.1
T. B.	7.6	6.5	5.9	5.9	6.1	6.3	6.3
M. M.	7.0	5.6	5.5	5.5	5.6	5.6	5.6
P. T.	7.2	6.1	5.3	5.3	5.4	5.5	5.5

The solid foods, French fried potatoes, apple, and the semisolid food, ice cream gave rise to little acid. When these foods were eaten, there was only a

TABLE III
pH OF PLAQUE MATERIAL AFTER EATING FRENCH FRIED POTATOES

SUBJECT	BEFORE EATING	5 MIN.	10 MIN.	15 MIN.	20 MIN.	25 MIN.	30 MIN.
J. L.	6.8	6.8	6.8	7.0	7.2	7.2	7.2
F. T.	7.5	6.6	6.4	7.1	7.3	7.1	7.0
T. B.	7.2	6.9	6.9	7.4	7.4	7.2	7.0
M. M.	7.1	6.4	6.7	6.8	7.0	7.0	7.1
P. T.	7.0	6.4	6.6	6.8	6.6	6.8	6.8

relatively slight fall in the pH so that after 5 to 10 minutes, pH readings in the vicinity of 6 were recorded for most subjects. Thereafter, the pH of the plaques rose steadily and usually reached normal levels in 20 to 25 minutes. Table III presents the findings for French fried potatoes which are representative of this group. The results for apples which gave rise to more acid than the ice cream or potatoes are given in Table IV.

TABLE IV
pH OF PLAQUE MATERIAL AFTER EATING APPLE

SUBJECT	BEFORE EATING	5 MIN.	10 MIN.	15 MIN.	20 MIN.	25 MIN.	30 MIN.
J. L.	6.8	5.9	6.0	6.3	6.9	7.0	7.0
F. T.	7.2	5.5	6.4	6.7	6.7	6.8	7.3
T. B.	7.3	6.6	7.0	7.0	7.0	6.9	7.1
M. M.	7.0	6.9	5.9	6.5	6.5	6.9	7.2
P. T.	7.2	6.8	6.6	6.9	6.8	6.8	7.2

Little acid was produced from the beverages. Typical findings are given in Table V. The acid production corresponds to that for French fried potatoes and apple, showing only a slight fall in the pH and a fairly rapid return to the normal levels. Comparison of the results listed in Table V with those in Table I shows a considerable difference between acid production in the plaque from beverages drunk in a normal way and from the use of a 20 per cent glucose rinse. This difference may occur because, during drinking, contact of the carbohydrate-containing beverages with the surfaces of the teeth is of a lower order than is the case with a glucose rinse deliberately held in the mouth for several minutes.

TABLE V
pH OF PLAQUE MATERIAL AFTER (CARBONATED) COLA BEVERAGE

SUBJECT	BEFORE EATING	5 MIN.	10 MIN.	15 MIN.	20 MIN.	25 MIN.	30 MIN.
J. L.	7.2	5.4	6.2	6.8	6.7	7.0	7.0
F. T.	6.8	6.1	6.0	6.8	6.7	6.7	6.7
T. B.	7.5	6.7	6.0	6.6	6.8	6.7	6.8
M. M.	7.2	6.8	6.7	6.8	6.8	6.8	6.9
P. T.	7.0	6.6	6.5	6.4	6.5	7.0	7.1

It is of interest to note that, with the exception of one subject, the pH of the plaque was not significantly depressed even for a short period either by the acid contained in the acidulated beverages or bacterial fermentation of the contained sugar (Table V). That no acid reaction had occurred in the plaque

prior to the 5-minute reading is indicated by the fact that since the time occupied in drinking the beverages was from 2 to 3 minutes, the first test measurement (5 minutes after the commencement of drinking) was actually made within 2 or 3 minutes of finishing the drinking of the beverages. Possibly the inherent acidity of the beverages prevented their ready fermentation by bacterial enzymes.

TABLE VI
AMOUNTS OF LACTIC ACID FORMED IN SALIVA AFTER THE CONSUMPTION OF DIFFERENT CARBOHYDRATE FOODS

FOOD	MICROGRAMS OF LACTIC ACID PER ML. OF SALIVA						
	BEFORE EATING	AFTER EATING					
		5 MIN.	10 MIN.	15 MIN.	20 MIN.	25 MIN.	30 MIN.
Fig sandwich cookie	22	1,275	1,840	1,380	615	240	110
Toffee	28	1,300	1,560	1,150	590	232	86
White bread butter and jam	20	950	1,425	980	395	182	72
Ice cream	32	1,350	1,250	665	184	57	30
French fried potatoes	21	1,030	1,150	510	174	56	21
Apple	28	1,120	900	420	256	80	25
Carrot	24	350	110	32	12	12	12
Cola beverage	18	980	1,080	420	170	12	10
Orange juice (canned)	30	805	495	256	100	50	20

Table VI presents the findings for lactic acid in saliva collected at intervals after eating the test foods. Since lactate was detectable only in ice cream and then only in small quantities, lactic acid in the foods can have had no influence on the findings in saliva. The results show a general direct relationship between the lactic acid figures and the plaque acidities. Lactic acid in saliva was highest and lasted longest with fig sandwich cookies, toffee, and bread and jam. Ice cream, French fried potatoes, apples, and sweetened beverages all gave relatively high concentrations of lactic acid within 5 or 10 minutes but as with plaques this acid production was of short duration.

DISCUSSION

The demonstration that there are considerable differences in acid formation in plaques following the consumption of different carbohydrate foods and beverages suggests that there might be parallel differences in the caries-producing capacities of these foods. This indicates that plaque measurements of the sort we have made could be used as a means of differentiating between the caries-producing potentialities of foods. That the measurements are useful for this purpose is indicated by the similar results obtained[8, 11] when the same foods were tested for caries-producing potentialities by measuring intraoral food retention and fermentation by oral bacteria. Support for the idea can also be drawn from animal experiments[12, 13] in which liquid carbohydrate foods which gave the least acid production in our plaque tests were shown to cause much less caries than equivalent amounts of carbohydrate fed in solid form.

On the other hand, there are reasons why reservations must be maintained as the value of the method we used. A very basic one is that the reliability of pH measurements in plaques has itself been questioned by Forscher and Hess.[14]

Therefore, in spite of the promising possibilities brought out by this study in respect to the use of plaque acidity for estimating the caries-producing potentialities of foods, it seems that refinements of technic or modifications of the approach must be made before the desideratum of a completely reliable method can be reached.

In view of the emphasis which is commonly placed by dentists on sugar-containing beverages as a cause of caries, the low and transient acidities produced by them in plaques need comment. These acidities were of the lowest order, being essentially the same as those produced by apples or fruit juice. This suggests a conclusion contrary to the common belief which possibly has originated from the assumption that the effects produced in the plaque by sugar-containing beverages will be similar to those produced by a glucose rinse. That sugar in liquid form is in fact likely to produce less caries is indicated by the findings of a number of investigators[1, 2, 3, 4, 7] who have shown that clearance of sugar from the mouth is much more rapid when it is consumed in liquid rather than in solid form. It is also supported by the animal experiments of Anderson[12] and Haldi[13] which, as mentioned above, showed that much less caries was produced in animals by liquid than by dry sugar.

SUMMARY

The production of acids in the dental plaque was studied after the ingestion of seven solid and three liquid carbohydrate foods. It was found that wide differences occurred between different foods and that the production of acids from sweetened beverages was generally lower than for certain solid foods. These observations were, to some extent, confirmed by the study of acid production in the saliva.

REFERENCES

1. Volker, J. F., and Pinkerton, D. M.: Some Observations Upon the Clearance of Sugar From the Mouth, *J. D. Res.* **26**: 9, 1947.
2. Boyd, L. J., and Speer, F. D.: The Concentration of Sugar in the Saliva After Ingestion of Carbohydrate-containing Substances, *Bull. New York M. Coll.* **13**: 104, 1950.
3. Haggard, H. W., and Greenberg, L. A.: The Concentration of Sugar in the Saliva After Ingestion of Carbohydrate Substances, *Dent. Surv.* **25**: 1788, 1949.
4. Lundqvist, C.: Oral Sugar Clearance. Its Influence on Dental Caries Activity, *Odont. Rev.* **3**: Supp. 1, 1952, C. W. K. Gleerup, Lund.
5. Ericsson, Y.: On the Salivary Amylase and Its Significance in the Caries Process, *Acta odont. scandinav.* **9**: 89, 1951.
6. Goldberg, H. J. V., and Bibby, B. G.: Unpublished data.
7. Bibby, B. G., Goldberg, H. J. V., and Chen, E.: The Evaluation of the Caries- producing Potentialities of Various Foodstuffs, *J. A. D. A.* **42**: 491, 1951.
8. Fosdick, L. S., Campaigne, E. E., and Fancher, O. E.: Rate of Acid Formation in Dental Caries. The Etiology of Dental Caries, *Illinois Dent. J.* **10**: 85, 1941.
9. Hartles, R. L., and McClean, N. D.: A Note on the Rapidity of the Production and Removal of Lactic Acid Following the Introduction of a 10% Glucose Solution Into the Mouth, *Brit. Dent. J.* **85**: 63, 1953.
10. Barker, S. B., and Summerson, W. H.: Determination of Lactic Acid in Biological Material, *J. Biol. Chem.* **138**: 535, 1942.
11. Ludwig, T. G., and Bibby, B. G.: In press.
12. Anderson, E. P., Smith, J. K., Elvehjem, C. A., and Phillips, P. H.: Dental Caries in the Cotton Rat. X. The Effect of the Fluidity of the Diet, *J. Nutrition* **35**: 371, 1948.
13. Haldi, J., Wynn, W., Shaw, J. H., and Sognnaes, R. F.: The Relative Cariogenicity of Sucrose When Ingested in the Solid Form and in Solution by the Albino Rat, *J. Nutrition* **49**: 295, 1953.
14. Forscher, B. K., and Hess, W. B.: The Validity of Plaque pH Measurements as a Method of Evaluating Therapeutic Agents, *J. A. D. A.* **26**: 19, 1954.

FURTHER OBSERVATIONS UPON THE CARIES-PRODUCING POTENTIALITIES OF VARIOUS FOODSTUFFS

T. G. LUDWIG,* B.D.S., M.S., AND B. G. BIBBY, B.D.S., PH.D., D.M.D.

Eastman Dental Dispensary, Rochester, N. Y.

OF the various attempts which have been made to establish the relative caries-producing capacities of different foods, only two[1, 2] have been applied to any considerable numbers of foodstuffs. One of these[1] with which we are concerned at this time, postulated that the caries-producing potential of a food could be determined by multiplying the quantity of the food retained upon the teeth after ingestion by the amount of acid produced on fermentation of the food in saliva. From this study, a tentative grading of 100 common foods was offered. However, since the ratings made in these tests were based upon the results obtained from only one or two subjects, it was stressed that confirmatory studies were needed. Subsequently, 50 foodstuffs were tested[3] in 4 or 5 subjects. Although the figures obtained were different, the foods retained essentially the same positions in the scale of caries-producing potentials. Incidentally, when similar foods were tested by Lundqvist[2] using a different procedure, they also fell in much the same order.

As a possible contribution toward increasing the reliability of laboratory determinations of decalcification potentials, this paper considers several variables which may influence the results and attempts to draw conclusions from a detailed study of a few representative foods.

A. Acid Production From Different Foods.—

In our previous studies[1, 3] the measurements of acid production were made with saliva collected on different days from a single subject. Therefore, day-to-day variations in the acid-producing capacity of saliva, which subsequent tests showed to be quite considerable, may have influenced the findings. To reduce this factor to a minimum, the acid production from the foods used in the present study were all made at one time in aliquots of saliva taken from a single saliva pool. Paraffin stimulation was used to collect the saliva from several individuals, after which it was pooled and refrigerated until required. In determining acid production, a 3 gram portion of the test food was emulsified in 100 ml. of distilled water and from this mixture two 5 ml. samples were taken and placed into sterile test tubes. Five milliliters of pooled saliva were then added to each sample. One of these was immediately

Received for publication Sept. 27, 1955. Revised by authors March 14, 1956.

Based on thesis of senior author submitted in partial fulfillment of requirements for M.S. (Dental Science) University of Rochester, 1954.

These studies were initiated under a grant from the National Institutes of Health and continued with the support of the American Bottlers of Carbonated Beverages.

*Present address: Dental Clinic, Upper Willis Street, Wellington, New Zealand.

titrated with N/10 sodium hydroxide. A Beckman potentiometer was used to determine the end point of pH 8.5 which was selected for this titration. The other sample was incubated at 37° C. for four hours, after which it was titrated in the same way. The difference between the first and second titration represented the acid formed by salivary fermentation. To obtain a mean value of each food, the test was repeated 3 times on different days, using different salivary pools.

The quantities of acid produced from the test foods when incubated in saliva and other findings which will be referred to later are listed in Tables I and II. The greatest quantity of acid was formed from milk chocolate.

TABLE I

ACID PRODUCTION AND FOOD RETENTION FOR SOME SOLID CARBOHYDRATE FOODS

FOOD	TOTAL CHO (%)	CHO RETAINED (MG.)	FOOD RETAINED (MG.)	4 HOURS ACID FORMATION (ML. 0.1 N)
Fig sandwich cookie	70	560	800	1.2
Date	77.5	393	507	1.6
Milk chocolate	50	185	370	2.1
Ice cream	17	72	424	1.6
Shortbread cookie	59.5	220	370	1.3
Toffee	90	250	277	1.8
Salted cracker	70	240	343	1.2
Oil sprayed cracker	71	220	310	1.2
Chocolate pudding	35	105	300	1.3
White bread	49	92	188	1.8
Apple	17.5	40	229	1.0
Carrot (fresh)	9.5	7	74	1.2

TABLE II

ACID PRODUCTION AND FOOD RETENTION FOR SOME BEVERAGES

BEVERAGE	TOTAL CHO (%)	CHO RETAINED (MG.)	FOOD RETAINED (MG.)	4 HOURS ACID FORMATION (ML. 0.1 N)
Pineapple juice (canned)	13	39	300	1.2
Orange soda (uncarbonated)	10	24	240	1.2
Cola (uncarbonated)	11	24	218	1.1
Orange juice (canned)	11.5	27	235	1.0
Cola (carbonated)	10.5	25	238	1.0
Orange soda (carbonated)	10.5	23	219	1.0
Orange juice (fresh)	8.5	15	176	1.2
Grape soda (carbonated)	11	23	209	1.0

Successively, smaller quantities were formed from such dissimilar foods as toffees, white bread, dates, and ice cream. The acid production values for beverages were low, much below that for the solution of toffees, which might have been expected to give similar figures. The amount of acid formed from

a particular food did not seem to depend solely upon the percentage carbohydrate content of the food, since more acid was formed from milk chocolate (50 per cent carbohydrate) than from toffees (90 per cent carbohydrate) or fig sandwich (70 per cent carbohydrate).

B. Variations in Food Retention From Time to Time.—

The possibility that there could be time-to-time variations in food retention in the mouths of test subjects has an obvious bearing on the establishing of retention values for any food.

To determine the extent of such variations, tests were made by having subjects ingest similar portions of the same food twice daily on 5 different days during a period of three weeks and measuring the quantity of food retained in each instance. Two commercial beverages and a doughnut were used for this purpose.

In determining the quantity of food retained in the mouth after eating, essentially the same methods as those described by Bibby, Goldberg, and Chen[1] were used except that the carbohydrate determinations were made by the anthrone method described by Morse[4] and by Morris.[5]

TABLE III

DAY-TO-DAY VARIATIONS IN FOOD RETENTION IN ONE INDIVIDUAL

FOOD	TIME	FOOD RETAINED (MG.)				
		DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Doughnut	10:30 A.M.	450	480	565	516	600
	11:30 A.M.	475	492	500	575	565
Cola beverage	10:30 A.M.	310	228	255	300	295
	11:30 A.M.	315	276	285	275	283
Orange soda	10:30 A.M.	251	277	210	360	255
	11:30 A.M.	319	245	245	360	228

Table III shows the variation occurring from time to time in food retention for one individual.

It was found that in estimations made at different times during the same day, results were usually of the same order. However, more widely spaced estimations, for example those made at weekly intervals, were subject to much greater variation. The reasons for this greater variation are not known. However, factors such as variations in the composition of different batches of a particular food, in the test subjects' eating habits, and in the rate of flow of his saliva may be responsible. The retention values obtained over a three-week period for doughnuts (Table III) ranged from 450 to 600 mg. and for a carbonated beverage, from 210 to 360 mg. These variations are of a high order and suggest the need of caution in interpreting results of food retention measurements.

Later studies show that the extent of the variations could be reduced by increasing the amount of the test food eaten so that complete "saturation" of

the mouth with the food would be insured, by adhering to a rigid time schedule for clearance procedures, and by using test subjects well acquainted with the difficulties in the experimental procedures.

C. Variation in Food Retention From Subject to Subject.—

Unless foods fall in relatively the same order when tested in different mouths, the use of retention figures would have little value in assessing the caries-producing potentialities of foods.

Using the same technics as above, repeated retention tests were made on 5 subjects, using 10 different foods to determine the nature of the variations in retention between different individuals. In these tests it was found that for any particular food some subjects gave retention values of a considerably higher order than did others (Table IV). However, the individuals who gave high values for one food gave relatively high values for all of the foods and, similarly, the subjects who gave low values for one food gave relatively low values throughout. Milk chocolate, cookies, and ice cream gave the highest values for most of the subjects, while the lowest values were given by white bread, bananas, apples, and beverages.

TABLE IV

VARIATIONS IN FOOD RETENTION IN DIFFERENT SUBJECTS

FOOD	SUBJECT A (MG.)	B (MG.)	C (MG.)	D (MG.)	E (MG.)
Ice cream	426	504	308	196	32
Milk chocolate	271	429	353	177	71
Shortbread cookie	223	332	433	240	59
Chocolate pudding	188	403	368	216	69
White bread	186	188	127	155	38
Oil sprayed cracker	123	237	117	218	25
Salted cracker	120	213	203	79	22
Banana	85	160	184	169	38

The comparison of foods in different subjects demonstrated that differences in retention between individuals are large. It also offered somewhat doubtful support for our earlier impression that foods giving a high retention value for one person would be retained in large quantities by the others. Whether there are patterns of variability between individuals in the retention of different foods has yet to be established.

D. Retention in Different Areas of Mouth.—

The extent to which foods taken into the mouth are retained on the teeth or elsewhere has not been determined. To test this, acrylic appliances were constructed for a test subject to cover the teeth and to afford a tight seal at the gingival margins. Test foods were eaten in the usual way, but on the completion of eating, instead of rinsing the mouth in the normal manner, the acrylic appliances were placed over the teeth so that the food adhering to the teeth would be held in place. The mouth was then thoroughly rinsed with distilled water. These rinsings were collected and contained the food which

had been retained upon the oral mucous membranes. Thereafter, the acrylic appliances were removed from the teeth, which were cleaned by use of an atomizer spray, and the mouth was rinsed again with distilled water. These rinsings were collected and pooled and to them were added the washings from the inside of the acrylic appliance. This total sample represented food which had been retained on the surfaces of the teeth. Carbohydrate determination on these two samples made it possible to calculate separately the amounts of the different foods held on the mucous membranes and on the teeth.

It was found (Table V) that some foods were retained in approximately equal proportions upon the teeth and on the mucous membranes; while for others a much higher proportion of the retained food adhered to the mucous membranes than to the teeth. In the former class were shortbread, cookies, dates, and milk chocolate. In the latter class were white bread, ice cream, apples, and sweetened beverage, although the difference was much more pronounced for apples and beverages than for the other two foods.

TABLE V

AREAS OF RETENTION IN THE MOUTH FOR DIFFERENT CARBOHYDRATE FOODS

FOOD	TOTAL CHO (PER CENT)	RETENTION ON MUCOUS MEMBRANE (MG.)		RETENTION IN TEETH (MG.)	
		CHO	FOOD	CHO	FOOD
Shortbread cookie	65	250	385	200	308
Date	77.6	250	322	200	258
Milk chocolate	50	160	320	150	300
Ice cream	20	60	300	30	150
Apple	20	50	250	2.5	12.5
White bread	50	62.5	125	40	80
Cola beverage	10	20	200	4.5	45

Since a food which adheres to the tooth surface is almost certainly more likely to participate in fermentation and acid production in the dental plaque than those persisting on the mucous membranes, the site of retention for a particular food represents a factor which should be considered in assessing its caries-producing properties.

E. Retention of Liquid and Solid Foods.—

The extremely low "decalcification potentials" given by sweetened beverages in our earlier study[1] suggested the desirability of confirming these findings and comparing them with some representative solid foods. For this purpose the retention of 12 solid carbohydrate foods and 8 sweetened beverages was determined by the usual method. The figures obtained are shown in Tables I and II. Additional comparisons can be found in Table III. As a whole, the retention figures for the solid foods are higher than those for the liquids. This is particularly obvious if the comparison is limited to the retention of the carbohydrate content of the food rather than the retention of the whole food. If this is done, all the solid foods, with the exception of carrot, give higher carbohydrate retention than the beverages.

DISCUSSION

It would appear at this stage that the assessment of the caries-producing capacities of foodstuffs by the methods of Bibby, Goldberg, and Chen[1] are subject to several limitations. The greatest difficulties met in making measurements of this type are in the accurate determination of food retention in the mouth. Not only are the analytical procedures involved subject to relatively wide sources of error, but variations in the retention of any one food occur from time to time in the same individuals. Although we have to some extent been able to substantiate the caries-producing ratings of different foods made by Bibby, Goldberg, and Chen, we feel that the significance of these findings should be re-evaluated. The use of a carbohydrate retention value rather than total food retention would seem to be indicated for calculations of decalcification potentials of certain, if not all, foods. If this is not done, calculations for foods, such as beverages with a low carbohydrate content, are unduly elevated by including as base for calculation the 90 per cent water content, whereas results for foods, such as toffee, are influenced only to the extent of the 10 per cent of other substances contained.

In addition, the fact that certain foods appear to be retained in much greater proportions upon the teeth themselves while others are retained chiefly upon the oral mucous membranes may considerably affect the amount of acid production from these foods on the tooth surface. The methods previously used do not seem well suited to permit assessment of this factor.

In addition to the difficulties associated with the measurement of food retention, the significance of the values obtained for acid production of the foods in salivary samples must be questioned. Apart from questions which might be raised as to methods in determining acid formation, it is doubtful whether the values obtained truly reflect the fermentability of the foods in the mouth. The effects produced by possible inhibiting[6] or stimulating[7] substances contained in the foods may differ considerably between the mouth and salivary samples in test tubes; and it is almost certain that the difference in environmental conditions[8] between the mouth and test tube will affect the rate and type of acid production from these foods. Moreover, it has been suggested[9] that the penetration into the dental plaque of the various carbohydrates contained in different foods may vary so that some will be more readily available for fermentation than others, and it is obvious that the significance of this factor cannot be assessed by in vitro measurement. These considerations may be even more pertinent in relation to the effects of foods on the progress of caries in existing cavities.

These conclusions suggest the desirability of attempting to assess the cariogenic capacities of different foods by measuring the degree and duration of acid production in dental plaques or cavities after eating. Preliminary studies will have been undertaken in these areas and will be reported subsequently.

SUMMARY

Using 20 representative solid and liquid carbohydrate foods, a study was made of some of the variables which must be considered when laboratory comparisons are made of the caries-producing potentials of foods. It was found that considerable variations in food retention occur between different subjects and from time to time in the mouth of the same subject, that for some foods retention on the mucous membranes exceeds that occurring on the teeth, and that the carbohydrate of liquid foods is retained in the mouth in smaller quantities than that of solid foods.

REFERENCES

1. Bibby, B. G., Goldberg, H. J. V., and Chen, E.: Evaluation of the Caries-producing Potentialities of Various Foodstuffs, *J. A. D. A.* **42**: 491, 1951.
2. Lundqvist, C.: Oral Sugar Clearance. Its Influence on Dental Caries Activity, *Odont. Rev.* **3**: Supp. 1, 1952, C. W. K. Gleerup, Lund.
3. Ludwig, T. G., Dewar, M. R., and Bibby, B. G.: Further Observations Upon the Caries-producing Potentialities of Various Foods, Proc. I. A. D. R., *J. D. Res.* **32**: 665, 1953.
4. Morse, E. E.: Anthrone for Estimations of Sucrose in Low Concentrations; *Ind. Eng. Chem. Anal. Ed.* **19**: 1012, 1947.
5. Morris, D. L.: The Determination of Carbohydrate in Biological Fluids by the Anthrone Method, *Science* **107**: 254, 1948.
6. Guillement, R.: 1936 cited G. W. Monier-Williams, Copper and Fermentation. Trace Elements in Food, London, 1949, Chapman & Hall, Ltd.
7. Wooley, D. W.: Effect of Manganese Upon Growth of Lactic Acid Bacteria, J. Biol. Chem. **140**: 331, 1941.
8. Elsden, S. R.: The Enzymes. **2**: Part 2, Chap. 65, p. 791, New York, 1952, Academic Press.
9. Joint report of Council on Dental Health and Council on Dental Therapeutics, Sugar and Dental Caries: The Effect on the Teeth of Sweetened Beverages and Other Sugar-containing Substances, *J. A. D. A.* **47**: 387, 1953.

THE EFFECT OF ALLOXAN DIABETES ON CARIES INCIDENCE IN THE ALBINO RAT

MARTIN S. NICHOLS AND JAMES H. SHAW

Harvard School of Dental Medicine, Boston, Mass.

AS early as 1882, a clinical impression of a relationship between diabetes mellitus and dental caries was advanced; Magitot[1] reported that the teeth of diabetics had an unusual susceptibility to dental caries. Thereafter, in the period prior to the utilization of insulin for diabetic control, numerous clinical reports about the incidence of dental caries in diabetics have been recorded. Grunert[2] postulated that individuals, formerly caries-free, who suddenly showed a high susceptibility to caries, should be suspected of having diabetes. Lederer[3] reported that the teeth of diabetics were predisposed to dental caries. Misch[4] maintained that caries progressed more rapidly in diabetic individuals, and Zilz[5] reported a sudden onset of caries in noncontrolled diabetics. Fleischman[6] observed no difference in caries-susceptibility between diabetics and nondiabetics. As clinical impressions, these reports all suffer from the failure to evaluate comparable diabetic and nondiabetic groups.

Beginning shortly after the introduction of insulin for the control of diabetes, Boyd and his associates[7-13] conducted a series of experiments in which they demonstrated the arrest of active carious lesions and a reduced rate of initiation of new carious lesions in diabetic children who were maintained either on a strict dietary regimen alone or in conjunction with insulin control. These workers established the fact that the dietary restrictions which are commonly a part of diabetic control exert a protective effect against dental caries.

Cohen[14] studied 27 young diabetics, on dietary and insulin control, and found no greater immunity to dental caries in them as compared to nondiabetics; moreover, his data suggest that the reverse is true and that there may be a steady increase in caries-susceptibility among diabetics after age 21. However, the group studied ranged in age from 6 to 30, with the result that there were very few subjects at each yearly age for comparison with normal individuals. Ziskin,[15] in a study of 94 diabetics at a diabetic camp, found no difference in the decayed, missing, and filled (DMF) count of this group

---

The material on which this paper is based was submitted as a thesis by the senior author in partial fulfillment of the requirements for the degree, Doctor of Dental Medicine.

The studies in this report were supported to a large extent by grant-in-aid No. A-577 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service. We are indebted to Merck & Company, Inc., Rahway, New Jersey, for generous supplies of B-complex vitamins.

Presented at the Thirty-third Annual Meeting of the International Association for Dental Research, Chicago, Ill., March, 1955 (J. D. Res. 34: 716, 1955. Abst.)

Received for publication Sept. 12, 1955.

as compared to a healthy group. Hinson[16] reported no significant difference between the caries incidence of diabetic children at the Joslin diabetic summer camp and that of a random sample of Newton school children. Since the subjects in all these studies of the caries-susceptibility of diabetics were under an appreciable measure of control by balanced insulin and dietary therapy, none of the studies adequately evaluate either the influence of noncontrolled diabetes on caries-susceptibility or the role of dietary control as a possible protective mechanism against dental caries.

Controlled studies regarding dental caries in adult diabetics have been minimal. Joslin, Root, White, and Marble,[17] on the basis of extensive clinical experience, stated that the high incidence of caries in adult diabetics is no greater that that in nondiabetics of comparable age. They had the impression that with the onset of diabetes during the second decade of life, many carious teeth are found, but that the process is arrested when control is brought about. Obviously, the study of this problem in human beings is difficult because of the wide age range among the diabetic population, the variations in the constitutional severity of the disease, the varying degree of control found among diabetics, the different methods utilized to achieve this control, and the lack of dietary standardization.

The use of alloxan for the production of a diabetic syndrome in laboratory animals provides an experimental approach to the study of this suggested relationship between diabetes and dental caries, with a minimum of variables with which to contend. Everett, Lal, and Phatak[18] reported on the effects of alloxan diabetes on the incidence of dental caries in the Syrian hamster. They stated that preliminary results indicated that alloxan-diabetic hamsters had substantially higher caries scores than normal controls when maintained on both cariogenic and noncariogenic diets. Nine and 4 diabetic animals, respectively, survived the experiment on these 2 diets.

The experiments described below were undertaken in an attempt to obtain more definitive information about the relationship of a noncontrolled diabetic syndrome to the incidence of tooth decay in caries-susceptible laboratory rats.

EXPERIMENTAL PROCEDURE

In the first experiment, 69 caries-susceptible albino rats were divided at 36 to 37 days of age into 2 groups according to sex and littermates. After a starvation period of 18 hours, 41 animals were injected intravenously in the femoral vein, with one dose of a 1 per cent aqueous solution of alloxan monohydrate at the rate of 48 mg. per kilogram of body weight. The remaining 28 animals served as controls.

All animals were housed in individual wire-bottom cages and were fed a cariogenic diet[19] ad libitum from weaning until sacrificed at 139 to 140 days of age, at which time dental caries was evident clinically. Blood sugar determinations were performed on all animals 1 week after injection of the alloxan. These determinations were done according to the micromethod of

Folin and Svedberg[20] on 0.1 ml. of blood drawn from the tail vein of the rat. Follow-up monthly determinations, including a determination during the week prior to sacrifice, were made on those animals exhibiting diabetes.

After sacrifice, the heads of all animals were stored in 95 per cent alcohol, the mandibles and maxillae stripped of investing tissue, and the molar teeth evaluated according to the method of Shaw, Schweigert, McIntire, Elvehjem and Phillips[21] for carious molars, carious lesions, and extent of carious lesions.

A second experiment was conducted which followed the design of the above experiment except in the matter of dietary intake. In this experiment, one group of 18 uninjected animals and one group of 31 injected animals were provided food ad libitum, while another group of 22 uninjected animals (hereafter referred to as the pair-fed control group) was offered weighed quantities of food equal to that consumed by injected littermates of the same sex.

RESULTS

In the first experiment, all 28 control rats survived the full experimental period. Of the 41 rats in the experimental group, 17 injected rats, including 6 diabetics which survived the first week postinjection, died prior to the conclusion of the experiment; 7 which never exhibited diabetes and 4 injected animals which exhibited diabetes for only a portion of the experimental period survived the duration of the experiment; and 13 injected animals both exhibited diabetes and survived the entire course of the experiment. All of these last-mentioned animals were considered to be diabetic on the basis of blood sugar values of greater than 315 mg. per cent for all monthly determinations. The terminal values for these animals were all greater than 400 mg. per cent. In contrast, the control animals had blood sugar values of less than 170 mg. per cent throughout the experiment.

In the tabulation of the results of this experiment, only those animals which were diabetic for the duration of the experiment and their control littermates were considered. Data for the control and diabetic groups are presented in mean values and standard deviations. Statistical comparisons between the 2 groups are all based on the "t-test" for significance of differences.

One striking finding was the development of cataracts by 77 per cent of the diabetic animals at some point during the course of the experiment. The occurrence of cataracts by sexes is tabulated in Table I. The incidence of cataracts in males and in females was approximately the same. No control animal developed any eye pathology.

The average final body weights of the rats are presented in Table I. The diabetic animals exhibited substantially lower body weights throughout the course of the experiment than those of the control animals. Likewise the final weights of the diabetic rats were significantly lower than those of their healthy littermates. The same observation obtained for male and female rats.

TABLE I
FINAL BODY WEIGHTS AND CATARACT INCIDENCE IN CONTROL AND DIABETIC RATS

GROUP	NUMBER OF RATS	NUMBER OF RATS WITH CATARACTS	FINAL BODY WEIGHT GM. AVG.	FINAL BODY WEIGHT GM. S.D.*	t†	p‡
Male						
Controls	3	0	344.8	25.2	40.6	<0.01
Diabetics	5	4	198.6	39.8		
Female						
Controls	12	0	203.2	26.2	25.3	<0.01
Diabetics	8	6	146.6	14.4		

*S.D.—standard deviation.
†$t = \frac{\text{difference between the means}}{\text{standard error of the difference}}$.
‡p = the probability that the difference between the means is due to chance.

The results of the caries evaluation are recorded in Table II. It is apparent that the male/female ratio in both diabetic and control groups was similar. The mean values for the number of carious molars, the number of carious lesions, and the extent of carious lesions were each slightly higher for the diabetic than for the control groups. However, these differences were not statistically significant.

TABLE II
DENTAL CARIES INCIDENCE IN CONTROL AND DIABETIC RATS

GROUP	NUMBER OF ANIMALS	NUMBER OF CARIOUS MOLARS AVG.	NUMBER OF CARIOUS MOLARS S.D.*	NUMBER OF CARIOUS LESIONS AVG.	NUMBER OF CARIOUS LESIONS S.D.*	EXTENT OF CARIOUS LESIONS AVG.	EXTENT OF CARIOUS LESIONS S.D.*
Controls	23 (9 ♂, 14 ♀)	10.5	0.7	21.0	1.8	80.9+	18.2+
Diabetics	13 (5 ♂, 8 ♀)	11.0	1.2	22.0	4.4	90.0+	29.0+
	t†	1.3		1.5		1.0	
	p‡	>0.1		>0.1		>0.1	

*S.D.—standard deviation.
†$t = \frac{\text{difference between the means}}{\text{standard error of the difference}}$.
‡p = the probability that the difference between the means is due to chance.

The frequency with which the production and maintenance of the diabetic state was achieved in the injected rats in the first experiment was not duplicated in the second experiment. Of the 22 injected rats, 8, including 3 diabetics, died during the experiment; 8 rats which never showed evidence of diabetes and 3 which had diabetes for part of the experiment survived the full period; 3 were maintained in a diabetic state throughout the entire experimental period. Two of the latter 3 animals had cataracts; all 3 had terminal blood sugar values greater than 400 mg. per cent. Since the dosage of alloxan and the experimental procedure were identical in the first and second experiments, the difference in the rate of production of the diabetic syndrome and in survival rates is not explainable. The caries evaluations for these diabetic animals and their control littermates were consistent with those observed in the first experiment and are not presented here because of the small numbers of eligible rats in each group.

Despite the small number of animals involved, this second experiment yielded valid data in regard to food consumption and its relation to body weight. These data are presented in Table III. The difference in body weight between diabetics and their pair-fed control littermates was similar to that in the first experiment. However, measurement of food intake indicates that the diabetics consumed substantially greater quantities of the experimental diet, with a mean value of 140 per cent the intake of the rats in the control group. Furthermore, data, on an additional 3 animals which were diabetic for approximately one-half of the experiment, are consistent for this diabetic period with the results just mentioned. However, with the return of the blood glucose levels in these rats to normal, their food intake became similar to that of their healthy controls and there was an accelerated rate of growth which permitted their body weights to approach those of their controls.

TABLE III
FOOD CONSUMPTION AND WEIGHTS OF DIABETIC VS. CONTROL GROUPS (EXPERIMENT 2)

LITTER	CONDITION	FINAL BODY WEIGHT GM.	FOOD INTAKE GM.	DIABETIC INTAKE / CONTROL INTAKE PER CENT
No. 1 ♂	Diabetic	255	2028	148
No. 1 ♂	Control	343	1373	
No. 2 ♂	Diabetic	205	2002	141
No. 2 ♂	Control	326	1416	
No. 3 ♂	Diabetic	177	1846	132
No. 3 ♂	Control	351	1398	
Mean Values				
	Diabetic	212	1959	140
	Control	340	1396	

DISCUSSION

Examination of the values obtained for the average number of carious molars reveals that this criterion for the evaluation of caries incidence approaches the maximal value possible. However, the values for the average number of carious areas and for the extent of carious lesions are considerably below those that would be found with complete carious destruction of all molars; hence, any difference in caries-susceptibility between the experimental and control groups would be manifest in differences in the latter parameters of tooth decay.

Since the diabetic animals had lower body weights than the normals, while consuming greater quantities of food throughout the experiment, it must be concluded that the difference in body weights between the 2 groups was based upon a metabolic inefficiency on the part of the diabetics in utilizing the food consumed. It is suggested that sugar lost in the urine of the diabetics may be the underlying mechanism of this metabolic inefficiency. The increased intake of food of the diabetic rats over that of the control rats, if anything, would favor higher caries scores in the diabetic group.

The results of the experiments described in this paper are seemingly at variance with those presented in abstract form by Everett, Lal, and Phatak.[18]

In the study done by the latter workers, diabetes was diagnosed by the presence of glycosuria. It is known that a transient renal tubular inflammation follows alloxan administration. Thus, it is conceivable that the renal clearance of glucose may be affected, and that glycosuria during this transient period may not be a true indication of a diabetic state. Repetition of the urinalyses would be vital for accurate diagnosis, but it is not known whether this was done. The surviving hamsters in the diabetic groups in this study were few in number and actual caries scores were not presented. Finally, no mention was made of the results of statistical analyses, so that the reported higher caries scores in the diabetic hamsters over those in normal hamsters may well not have been statistically significant if a high variation occurred within groups.

The alloxan-diabetic rats in this study exhibited many signs similar to those which characterize diabetes mellitus in the human being; they demonstrated polyphagia, low body weights, hyperglycemia, polyuria (not mentioned in the results), and a high incidence of cataract formation. Other observations[22] of glycosuria, ketosis, acidosis, and response to insulin have been described in alloxan-diabetic rats, suggesting a further relationship to human diabetes mellitus. The results of these experiments suggest that the alloxan-diabetes syndrome does not result in any striking increase in dental caries incidence in the caries-susceptible rat fed a cariogenic diet. The relevance of these data to man is unknown, largely because of the high cariogenicity of the diet and the uncertainty about the similarity of alloxan-diabetes in rats to diabetes mellitus in man.

SUMMARY

A diabetic syndrome was produced in caries-susceptible rats by the intravenous injection of alloxan monohydrate. The diabetic rats had high blood glucose levels, a high incidence of cataracts, a slow rate of growth, low adult body weights and an unusually high rate of food consumption.

No statistically significant difference was found between the number of carious molars, the number of carious lesions and the extent of carious lesions in the molar teeth of normal albino rats and their alloxan-diabetic littermates which were maintained on a cariogenic diet for 103 days.

REFERENCES

1. Magitot, E.: Memoire sur la valeur diagnostique dans le diabete sucre de la periostite alveola-dentaire, *Acad. Med.* 1882.
2. Grunert, A.: ub. Krankheitserscheinungen in der Mundhohle bein Diabetes, Therapeut. Winke f. Diabetiker, Berlinische Verlagsanst., Berlin, 1899, 29p.
3. Lederer, W. J.: The Relationship Between Dental and Systemic Disturbances, *New York M. J.* **89**: 65, 1909.
4. Misch, J., Editor: Lehrbuch der Grenzgebiete der Medizin und Zahnheilkunde, Leipzig. 1922, F. C. W. Vogel, vol. I, p. 124.
5. Zilz, J.: Statistical Observations on Diabetes and Pyorrhea Alveolaris, *D. Cosmos* **57**: 102, 1915.
6. Fleischman, J.: Die Temporare Disposition zur Karies, *Oesterr-Zeitsch. f. Stomatol.* Part 5, 1913.
7. Boyd, J. D.: The Use of Standard Diet Formulas in the Control of Juvenile Diabetes Mellitus, *J. A. M. A.* **87**: 1020, 1926.

8. Boyd, J. D., and Drain, C. L.: The Arrest of Dental Caries in Childhood, *J. A. M. A.* **90**: 1867, 1928.
9. Boyd, J. D., Drain, C. L., and Nelson, M. V.: Dietary Control of Dental Caries, *Am. J. Dis. Child.* **38**: 721, 1929.
10. Boyd, J. D.: Nutrition as It Affects Tooth Decay, *J. Am. Dietetic A.* **18**: 211, 1942.
11. *Idem:* Long Term Prevention of Tooth Decay Among Diabetic Children, *Am. J. Dis. Child.* **66**: 349, 1943.
12. *Idem:* Prevention of Dental Caries in Late Childhood and Adolescence, *J. A. D. A.* **30**: 670, 1943.
13. *Idem:* Dental Caries as Influenced by Fat Versus Carbohydrate in the Diet, *Am. J. Dis. Child.* **67**: 278, 1944.
14. Cohen, M. M.: Clinical Sudies of Dental Caries Susceptibility in Young Diabetics, *J. A. D. A.* **34**: 239, 1947.
15. Ziskin, D. E., Siegel, E. H., and Loughlin, W. C.: Diabetes in Relation to Certain Oral and Systemic Problems. I. Clinical Study of Dental Caries, Tooth Eruption, Gingival Changes, Growth Phenomena and Related Observations in Juveniles, *J. D. Res.* **23**: 317, 1944.
16. Hinson, H. L.: Dental Disease in Diabetic Children, Thesis for D.M.D. degree, 1952, Harvard School of Dental Medicine Library.
17. Joslin, E. P., Root, H. F., White, P., and Marble, A.: The Treatment of Diabetes Mellitus, Philadelphia, 1952, Lea & Febiger, 446 pp.
18. Everett, F. G., Lal, D., and Phatak, N. M.: The Effects of Alloxan Diabetes on the Incidence of Dental Caries in the Syrian Hamster, *J. D. Res.* **28**: 653, 1949.
19. Shaw, J. H.: Carious Lesions in Cotton Rat Molars. I. The Role of Mechanical Factors Studied by the Extraction of Antagonistic Molars, *J. D. Res.* **26**: 47, 1947.
20. Folin, O., and Svedberg, A.: Micro Methods for the Determination of Non-Protein Nitrogen, Urea, Uric Acid and Sugar in Unlaked Blood, *J. Biol. Chem.* **88**: 85, 1930.
21. Shaw, J. H., Schweigert, B. S., McIntire, J. M., Elvehjem, C. A., and Phillips, P. H.: Dental Caries in the Cotton Rat. I. Methods of Study and Preliminary Nutritional Experiments, *J. Nutrition* **28**: 333, 1944.
22. Lukens, F. D. W.: Alloxan Diabetes, *Physiol. Rev.* **28**: 304, 1948.

INHIBITION OF ACID PRODUCTION OF SALIVARY SEDIMENT BY ETHERS, ESTERS, PHENOLS, AND ALCOHOLS

R. S. MANLY, PH.D., AND GLADYS HARGREAVES, M.S.

Tufts University School of Dental Medicine, Boston, Mass.

SINCE 1952 an empirical program of testing in this laboratory has involved a wide variety of chemicals which are routinely screened for their ability to inhibit acid production by salivary sediment. The experimental methods have already been described.[1] The first presentation concerned 350 substances containing a hydroxyl group which were selected from a group of 1,200 compounds tested.[2] Later a report was made on 186 amines that sometimes contained other organic groups such as ether, ester, alcohol, or phenol.[3] Additional substances have been tried since the previous report on alcohols, and now there are available for consideration 834 alcohols or phenols from a group of 2,400 compounds which have been studied. The purpose of this presentation is to describe the active substances to be found among the simplest of organic structures, those compounds containing only carbon, hydrogen, and oxygen, in which the oxygen is in the form of ether, ester, alcohol, or phenol linkages.

EXPERIMENTAL PROCEDURES

The procedures used in testing the majority of compounds differs slightly from that set forth previously.[1] Salivary sediment was obtained by centrifuging pooled, stimulated saliva for 20 minutes at 2,400 r.p.m. The subjects who contributed to the pool were selected to produce a pH differential of 1.5 units when the sediment was in contact with glucose buffer. Thirty microliters of the sediment was pipetted into a shaped nylon thimble which was pulled carefully against the active surface of a Beckman #4990-29 glass electrode. The sediment-coated electrode was placed in a solution containing .2 molar glucose, 0.01M $NaHCO_3$-CO_2 buffer, and 10 per cent of the pooled supernatant liquid from saliva.

Each cell contained 1 calomel and 2 glass electrodes, 1 of which was coated with sediment. The bare electrode read the pH of the solution and the coated electrode that of the sediment. The readings were recorded automatically on a Minneapolis-Honeywell strip chart recorder. After a steady state was attained with a control solution in the cell, the test solution was added for 20 minutes and then removed and replaced by a fresh control solution. The calculation of the per cent of activity is based on the pH differential of the

Supported by Grants from the American Chicle, Beech-nut Packing, Colgate-Palmolive, Lambert Pharmacal, Lever Brothers, and Procter and Gamble Companies and the Sugar Research Foundation.

This paper was presented in part at the International Association for Dental Research, March 18, 1954, French Lick Springs, Ind. (*J. D. Res.* 33: 672, 1954.)

Received for publication Aug. 23, 1955.

sediment during the first control period. The formula employed was as follows:

$\frac{B_2 + B_1 - 2S_2}{2B_1 - 2S_1}$ where B_1 = buffer pH at end of control period, B_2 = buffer pH at end of experimental period, S_1 = sediment pH at end of control period, and S_2 = sediment pH at end of experimental period.

This equation was derived empirically from a study of the influence of pH of solution on the pH of the sediment. This formula reduces to the ratio of pH differentials in case the buffer pH remains the same, i.e., is not influenced by hydrolysis of the test chemical. In most instances the solutions were titrated approximately to the pH of the buffer before test, and if no hydrolysis occurred the results by the above calculation differ from a ratio of differentials by no more than 2 per cent.

A similar formula was used for calculating "recovery" or the residual influence of the test chemical:

$\frac{B_3 + B_1 - 2S_3}{2B_1 - 2S_1}$ where B_3 is the pH of buffer at end of the second control period, and S_3 the pH of the sediment at the same time.

In nearly all instances the recovery values were the same or lower than the transient values. Recovery values give better indication of practical utility since they measure substantivity, and are unaffected by the buffer capacity of the test chemical. For this reason only recovery values will be reported.

RESULTS

To date we have tested 834 compounds that contain a hydroxyl group. Part of these have been tested both in water solution and in a 1:9 mixture of propylene glycol and water (10 per cent propylene glycol). All substances showing recovery values of 65 per cent or less have been tested in duplicate. Many of these compounds contained other groupings such as aldehydes, ketones, halogens, or amines, which might themselves be responsible for activity.

Our approach was to study first those substances which had least chance of being active, then those which appeared to be inactive per se could be admitted in the next chemical group to be studied. Table I presents information on the distribution of the inhibitory action among ethers, esters, and hydroxyl compounds, when all other functional groupings such as aldehydes, halogens, amines, sulphur derivatives, or unusual metals were eliminated. The figures in the body of the table refer to numbers of substances which permit salivary sediment to recover to the extent shown by the position in the vertical column. The recoveries are grouped from active toward inactive in 5 columns and the total compounds tested are given in the sixth. There were 34 ethers which contained no other functional group. None of these caused much inhibition, as shown by the absence of compounds capable of reducing recovery below 59 per cent. Ether linkage is not apt to impart inhibitory action.

A similar conclusion was drawn from the study of esters. There were 3 substances which caused inhibition greater than 40 per cent of a total of 46

TABLE I
DISTRIBUTION OF INHIBITION AMONG ETHERS, ESTERS, AND HYDROXYL COMPOUNDS CONTAINING NO OTHER FUNCTIONAL GROUPINGS

RECOVERY OF ACID FORMATION RATE (% OF CONTROL)	0-19	20-39	40-59	60-79	OVER 80	TOTAL
Ethers only	0	0	0	10	24	34
Esters only	1	0	1	15	29	46
Ethers and esters	1	0	4	32	74	112
Hydroxyl and ether and/or ester	13	34	36	97	263	443
Same without glycol	1	0	4	21	181	207
Same with glycol	12	34	32	76	82	236

compounds studied. The substance showing considerable activity was α-methyl benzyl acetate, the inhibitory action of which could have resulted from the presence of free alcohol in the ester, either as impurity in the ester or resulting from hydrolysis of the ester by sediment. The alcohol has inhibitory action which will be discussed later. There were 32 compounds which contain both ester and ether linkage and no other functional groups. When these were combined with ethers and esters, there was a total of 112 substances from which we could estimate the probability of the inhibitory substances among ethers and esters. Only one substance mentioned shows inhibitory effect greater than 60 per cent. This finding suggests that ether and ester groupings per se are not apt to be inhibitory, and it indicates that ethers and esters may be included among groups of compounds where the activity of some other functional group is being studied.

When hydroxyl compounds were selected, the findings were more encouraging. There was a total of 380 hydroxyl compounds, 63 of which were tested both in water and in 10 per cent propylene glycol. Many contained ether or ester groups but none had other functional groups. Forty-seven substances (11 per cent of the total tests) showed inhibition to a considerable degree. There were 207 substances tested in water and 236 tried in the presence of 10 per cent propylene glycol (except for 2 or 3 tests using 10 per cent triethylene glycol). The solvents alone were not inhibitory. In the absence of propylene glycol, there was 1 substance with inhibitory action greater than 60 per cent, whereas in the presence of solvent there was a total of 46 compounds (20 per cent) which possessed considerable inhibitory effect.

Of the 46 compounds tested in the presence of propylene glycol and showing recoveries of 30 per cent or less, there were 15 compounds which had also been tested without solvent. Twelve of these had recovery figures over 80. Two were between 60 and 79 in recovery and only 1 had a recovery between 40 and 59 per cent. This indicated propylene glycol enhances the inhibitory effect.

There were 9 compounds tested at 1 per cent concentration, both in water solution and in 10 per cent propylene glycol. These compounds are presented in Table II. In every instance the recovery was lower when the compound was tested in the presence of propylene glycol. Apparently the glycol has a synergistic action under certain circumstances. It may act as a penetrant to carry the test substance more rapidly or intimately into contact with the

microorganism or the enzyme systems, or it may have a subthreshold inhibitory action that can only be disclosed when some other inhibitor is present. Propylene glycol at 10 per cent concentration is just below that needed to be inhibitory.

TABLE II
EFFECT OF 10 PER CENT PROPYLENE GLYCOL ON INHIBITORY ACTION

NAME	RECOVERY (%) FROM CONTACT WITH COMPOUND AT 1% IN:	
	H_2O + 10% PG	H_2O
Phenylethyl alcohol	22	52
2-Ethyl-1,3-hexanediol	39	91
Sorbitol	89	62
1,4,7-Heptanetriol	56	137
2-Methopymethyl-2,4-dimethylpentanediol-1,5	66	112
Isobutyl alcohol	62	94
1,7-Heptanediol	72	106
Ethylene glycol monobutyl ether	97	114
1,4,7-Octanetriol	92	101

In the majority of instances, propylene glycol is beneficial because it allows for higher concentrations of chemical at the time of test. Thirteen inhibitory substances were selected from those tested in propylene glycol. Saturated solutions of each were prepared in water. To 100 ml. aliquots of each solution were added 10 ml. propylene glycol, 10 ml. glycerine, and 10 ml. water. Each of these solutions was again diluted 1:1 with buffer for test. The substances so studied were as follows:

2-(*p*-chlorophenoxy)ethanol
1-(*p-sec.*-butylphenoxy)-2-propanol
p,p'-sec.-Butylidene diphenol
p-tert.-Amyl phenol
p,p'-Isopropylidene diphenol
o-sec.-Amyl phenol
p,p'-Biphenol
1-(*o-sec.*-butylphenoxy)-2-propanol
p-phenyl phenol
2, 6-dimethoxy phenol
p-(benzyloxy) phenol
1, 3-diphenoxy propanol
benzyl carbitol

The above compounds are given in order of decreasing inhibitory effect. When tested at half saturation in propylene glycol, the first 4 compounds had recovery values of less than 50 per cent.

None of these compounds were sufficiently soluable in water to have appreciable inhibitory activity at 0.45 saturation. At such low concentrations of chemicals neither 5 per cent propylene glycol nor 5 per cent glycerine was able to enhance inhibition to any appreciable degree. The means of all 13 recovery values in glycerine and water were identical. The mean for propylene glycol was 88 per cent instead of 94 per cent. No recovery value was less than 77 per cent for compounds in water and glycerine. This indicates that

a major problem of producing inhibitory action from various substituted phenols is to obtain sufficient concentration of chemicals in solution. The advantage of 10 per cent propylene glycol arises more often from its ability to increase concentration than from a synergistic action.

The influence of solvent on the activity of phenol was studied at a variety of concentrations, in water, in 5 per cent propylene glycol, and in 5 per cent glycerine. The results are given in Table III. There is no indication of a synergistic influence of propylene glycol or glycerine on the inhibitory action of phenol.

TABLE III
EFFECT OF SOLVENT ON INHIBITION BY PHENOL

	CONCENTRATION AT TEST (%)	RECOVERY VALUE FOR PHENOL IN WATER SOLUTION CONTAINING:		
		GLYCOL	GLYCERINE	SOLVENT
Crystalline product	0.9	11	9	13
Liquid product	0.9	27	26	28
Crystalline product	0.09	90	82	103
Crystalline product	0.009	95	99	103

The alcohols were examined to determine whether there was any relationship between the number of carbon atoms, code units, and activity. Each alcohol, ester, or ether group was counted as 1 code unit. Monosubstituted alcohols would have 1 code unit. Disubstituted alcohols, monosubstituted alcohol-ethers, and alcohol-esters would contain 2 code units. The incidence of activity was high among compounds with 1 or 2 code units, was lower for compounds having 3 code units, and practically nil (6 per cent) for compounds having 4 code units or more. Inhibitory compounds generally had 3 or less code units.

When compounds having 1 to 3 code units were further segregated according to carbon count and types of functional groups, additional generalizations were observed. There were 62 monosubstituted alcohols containing no other active functional groups. Marked activity was observed only among those compounds containing 6 to 13 carbon atoms, inclusive. All other compounds had recoveries of 40 or greater. Forty-four per cent of the group having 6 to 13 carbon atoms showed activity. Among disubstituted alcohols containing no other functional group, there were 7 out of 36, or about 20 per cent, having recoveries of 39 or less, but when those with 6 to 13 carbon atoms were considered the incidence of inhibition was nearly 40 per cent. There were 37 monosubstituted alcohols containing 1 ether linkage, of which 27 per cent were inhibitory, but among those with 6 to 13 carbon atoms 36 per cent were active. Nearly all strongly inhibitory compounds had 1 to 3 code units and 6 to 13 carbon atoms. It seemed justifiable to exclude all other compounds in any consideration of the possible types of groupings which could impart activity.

The narrowed group of compounds were further separated into 3 groups —acyclic, alicyclic with or without acyclic groups, and aromatic with or without acyclic. There was 15 per cent incidence of inhibition among the acyclic group, 39 per cent among the aromatic series and 56 per cent among

the alicyclic compounds. This finding suggests that, in general, the alicyclic compounds are more apt to have activity than the aromatic compounds, which in turn are more apt to be inhibitory than the acyclic compounds.

There seemed to be no indication that phenols were any different in inhibitory properties than that of similar alcohols with the hydroxyl group on a side chain. Phenol, benzyl alcohol, phenylethyl alcohol, and 3-phenyl-1-propanol showed considerable activity, and the differences might be attributable to solubility. There is some indication that the 3-cyclohexenyl and cyclohexyl rings are likely to cause higher inhibitory properties than the phenyl group. This observation holds true for the carbitol and the carbethoxyethanol.

The bicyclo(2, 2, 1)-5-heptene ring differs from 3-cyclohexenyl ring by the presence of a methylene bridge. These 2 derivatives of methyl alcohol are highly active and the corresponding phenylmethyl alcohol is moderately active. Apparently the bicyclo ring augments inhibitory action as can be seen by comparing the low readings obtained with bicyclo-(2, 2, 1)-5-heptene-2-methyl carbitol and the inactivity of α-methylbenzylcarbitol. Probably there are other alcohol derivatives of 3-cyclohexene and the bicyclo heptene ring that deserve to be tested.

There were 8 compounds for which ortho- and parasubstitution on phenol could be compared. These compounds range in complexity from ortho- and paracresol to ortho- and paracyclopentenyl phenol and ortho- and para-*sec.*-butylphenoxy-2-propanol. There were no differences in activity in 7 out of 8 cases even though there were 2 instances in which the para derivatives were soluble to the extent of 1 per cent and the ortho compound was less soluble. This finding indicates a similarity in inhibitory action of ortho- and para-substituted compounds.

TABLE IV
COMPARISON OF INHIBITORY EFFECT OF ALCOHOLS, PHENOLS, AND THEIR CORRESPONDING CELLOSOLVES AND CARBITOLS

RADICAL	RECOVERY OF R-OH	R-O-CH_2. CH_2OH	R-(O-CH_2. CH_2) 2-OH
Phenyl	0	82	–
Benzyl	44	84	–
o-Cresyl	25	26	41
2-Methyl benzyl	64	99	93
o-sec.-butyl	10	27	–
Thymyl	33	26	–
Cyclohexyl	66	63	–
Cyclohexyl methyl	26	53	–
3-Cyclohexenyl-1-methyl	3	19	–
Bicyclo(2, 2, 1)-5-heptene-2-methyl	0	28	8

There were 10 comparisons possible between phenols and the corresponding oxyethanol or mono ether of ethylene glycol (Table IV). The oxyethanol derivatives averaged 17 per cent higher than the phenols for the recovery values. With phenol the recovery was zero at 1 per cent and was inactive at 1 per cent for the 2-phenoxy ethanol. For the remaining 9 substances the inhibitory effect of the oxyethanol derivatives was the same as that of the original alcohol in 3 cases and less active in 5 cases. In 3 instances 1 additional molecule of ethylene glycol was condensed to form the alkoxy ethoxy ethanol or the "carbitol" which on the average made no change in activity.

Table V contains information on inhibitory action of all hydroxyl compounds mentioned in Table I. This detailed information is of value to investigators who wish to compare their findings with ours with regard to specific compounds.

Compounds are divided first into 5 groups according to their recovery values. Next they are separated into 2 subgroups, based on the presence or absence of solvent during the test. Within each subgroup the compounds are listed in alphabetical order to enable a particular compound to be located with some ease.

DISCUSSION

During the first few months of testing all compounds were dissolved in water for study. This practice almost caused us to overlook the potentialities of hydroxyl compounds. Nearly 70 of these were studied in water solution without finding any which showed much inhibitory action. The poor solubility of some alcohols and substituted phenols led us to try the use of mixed solvents. Propylene glycol was selected because it was less viscous than glycerin and was tolerated by sediment at higher concentration than was isopropyl alcohol. This change of technic to make use of accessory solvents is responsible for the discovery of potentialities among the hydroxyl compounds. There is justification for using glycerine or propylene glycol as an accessory solvent since such vehicles are usually employed for obtaining desirable physical properties of tooth pastes.

Apparently these compounds have never been tried at sufficient concentrations by other workers for their potential value to be noted. Some investigators apparently assumed that useful substances should be effective at concentrations of 0.05 per cent or less,[4] and none seem to have used accessory solvents to increase solubility. Only 1 compound, inositol, is common to the series of compounds examined by Fosdick[4] and by us. Both groups of investigators are in agreement that it is inactive.

There are several inhibitory compounds which deserve consideration. Both eugenol and phenylethyl alcohol are of interest because of their possible employment as flavoring agents in oral preparations. Eugenol has been used as 1 component in zinc oxide–eugenol temporary filling materials. Eugenol might aid in inactivating acid production by organisms which remain in a cavity after the cavity preparation. We found that placement of a zinc oxide–eugenol filling had a depressing effect on the activity of sediment obtained from the saliva of 1 person for 2 or 3 days after the filling was placed.

The inhibitory action of hexylresorcinol at .1 per cent in 10 per cent propylene glycol raises an interesting question. It is employed in a proprietary mouthwash at 1 per cent concentration in the presence of glycerine. Instructions are given that the product be diluted with 1 or 2 parts of water before use. There is some possibility that this concentration could have an inhibitory influence on salivary sediment.

Sesamol is the methylene ether of 3, 4-dihydroxyphenol and is derived from sesame oil. The potential usefulness of sesamol cannot be estimated without knowledge of its toxicity relative to phenol. The same holds for

TABLE V

I. Recovery of 0 to 19 per cent

a. *Tested in 10 per cent propylene glycol solution*

Bicyclo (2, 2, 1)-5-heptene-2-methanol	3,5-Dimethylphenyl "Cellosolve"
(Bicyclo [2, 2, 1]-5-heptene-2-methyl) "Carbitol"	*n*-Hexyl "Carbitol"*
	Hexyl resorcinol**
o-sec.-Butyl phenol	Phenol*
3-Cyclohexenyl-1-methanol	Sesamol
3-Cyclohexenyl-1-methyl "Cellosolve"	2,5-Xylenol

II. Recovery of 20 to 39 per cent

a. *Tested in 10 per cent propylene glycol solution*

2-Allylphenyl "Cellosolve"	*n*-Butyl-tartrate
o-sec.-Amylphenol	2-Ethyl butyl "Cellosolve"
(Bicyclo [2, 2, 1]-5-heptene-2-methyl) "Cellosolve"	2-Ethyl-1,3-hexanediol*
	Guaiacol**
p-(2-Butenyl) phenol	*n*-Hexyl "Cellosolve"
Butenyl phenols (mixed)	Methyl amyl alcohol
4-*tert.*-Butylcyclohexanol	2,2′-Methylenediphenol
p,p′-sec.-butylidene diphenol	2,4′-Methylenediphenol**
1-(*p-sec.*-butylphenoxy)-2-propanol	2-Methylhexahydrobenzyl alcohol
1-(*p-tert.*-butylphenoxy)-2-propanol	Nonyl "Carbitol"
o-Cresol	2-Phenylethanol
p-Cresol	3-Phenyl-1-propanol
Cresyl "Cellosolve"	Thymol
Cyclohexyllactate	Thymol "Cellosolve"
1-Cyclohexyl methanol	3,3,5-Trimethylcyclohexanol
1,2-Decanediol	3-[2-(3,3,5-Trimethylcyclohexanol) isopropoxy]-2-propanol

III. Recovery of 40 to 59 per cent

a. *Tested in 10 per cent propylene glycol solution*

p-tert.,-amyl-phenol	1,4,7-Heptanetriol*
Benzyl alcohol*	*o*-Hydroxy-benzyl alcohol
Benzyl lactate	5-Hydroxy-2-hydroxymethyl-1,4-pyrone*
4-*tert.*-butyl-*o*-cresol	2-Methoxy-4-methylphenyl "Cellosolve"*
o-butylphenol	2-(2-[α-methylbenzyloxy]ethoxy) ethanol
2-*p*-(*tert.*-butylphenoxy) ethanol	1-Mono-*n*-butyrin
Cresyl "Carbitol"	1,2-Octanediol
1-Cyclohexyl methyl "Cellosolve"	2-Octyllactate
o-Cyclohexylphenol	*n*-Octyllactate
o-Cyclopentenylphenol	*N*-octyllactyl lactate*
p-Cyclopentenylphenol	Penolate
5-Decyne-4, 7-diol.-4,7-dimethyl*	Phenol, 4-allyl-2,6-dimethoxy
α,α-Dimethylphenylethyl alcohol	1-Phenoxy-2-propanol
p-Dodecylphenol	Sorbitol*
2-Ethyl butanol	2,4,7,9-Tetramethyl-5-decyne-4,7-diol
2-Ethylhexane-1, 4-diol	Triethylcarbinol
(4-Ethyl-1-methyloctyl) "Cellosolve"	
Eugenol	

b. *Tested in water solution*

4-*tert.*-Butylcatechol**	*n*-Hexyl "Carbitol"*
Carbitol*	2-Phenylethanol*

IV. Recovery of 60 to 79 per cent

a. *Tested in 10 per cent propylene glycol solution*

Ascorbic acid	2-Butanol, 3-butoxy*
Benzyl "Cellosolve"	2-Butanol, 3-ethoxy*

No marks = S/2, obtained by 1:1 dilution of saturated solution of the chemical in 20% propylene glycol.

* = 1 per cent.

** = 0.1 per cent, 0.01 per cent, or S/10.

*** = tested in 10% triethylene glycol (Dowanol 62B).

TABLE V—CONT'D

p,p'-Biphenol
1,3-Butanediol*
1,4-Butanediol*
n-Butyl alcohol*
Butyleneglycolphenyl ether
Butyllactate lactate
2-(*p-tert.*-Butylphenoxy)ethanol
p-tert.-Butylphenyl salicylate
α-Conidendrol
β-Conidendrol
Crotylphenyl "Carbitol"
Crotylphenyl "Cellosolve"
Cyclohexanol*
Cyclohexyl "Cellosolve"
1-Cyclohexyloxy-2-propanol
Decamethylene bisphenol*
2,2'-Diallyl-*p,p'*-biphenol
Di-*sec.*-amyphenol
7, 10-Dimethylhexadec-8-yne-7,10-diol
2,5-dimethyl-3-hexyne-2,5-diol
8,11-Dimethyloctadec-9, yne-8,11-diol
3-6-dimethyl-4-octyne-3, 6-diol*
Dipropylene glycol *n*-butyl ether*
2,5-Ditertiary butyl hydroquinone
1,2-Dodecanediol
Ethoxyethyl gallate
2-Ethyl-1-butanol
Ethylene glycol hydrogen butyrate
2-Ethylhexyl "Carbitol"
2-(2-Ethylhexyl) cyclohexanol
Ethyllactate lactate*
Phenylethylacetate
Polyethylene glycol 200 monolaurate**
Pyrogallol*
Phenol salicylate
Resorcinol Monoacetate*
Styrene glycol

2-Butanol, 3-methoxy*
2-Butoxymethyl-2,4-dimethyl-1,5-pentanediol
Ethyl salicylate
1,7-Heptanediol*
1,2,6-hexanetriol
Hexylene glycol*
n-Hexyl Lactyllactate
Hexyl Mandelate
(1-Hydroxycyclohexyl-1)-acetylene
1-Hydroxy-1-cyclohexyldiphenyl carbinol
Inositol
Isobutyl alcohol*
p-isopropylbenzyl alcohol
p,p'-Isopropylidenediphenol
Lactic acid, 2-(2-Methoxyethoxy) ethyl ester*
2-Methallyl lactate*
2-Methoxymethyl-2,4-dimethyl-1,5-pentanediol
α-Methyl benzyl alcohol
o-(3-Methyl-2-butenyl) phenol
Methyl *α*-D Glucoside
Methyllactate lactate*
4-Methyl-2-Pentanol
3-Methyl-1-pentyn-3-ol*
Methyl salicylate
1-Monopropionin*
α-Naphthyl "Cellosolve"
p-Nonyl-*o*-Cresol
Octyllactate
p-Pentadecylphenol
Phenol, *o*-isopentyl
Terpin hydrate
Tetrahydropyran-2-Methanol*
Tributyl citrate
Triethyl citrate*
d-Verbenol

b. *Tested in water solution*

Adonitol**
p-(Benzyloxy) phenol**
1-(2-Biphenyloxy)-2-propanol**
2-Butoxyethanol**
2-Butoxymethyl 1-2,4-dimethyl-1,5-pentadiol**
Cresyl "Cellosolve"**
Di-*sec.*-Butylphenol**
2,4-Di-*tert.*-Butylphenol**
Diethylene Glycol Monobutyl Ether*
Diphenyl-2,5-Hexyndiol**

1-Ethynyl Cyclohexanol-1**
Glyketal (2-methyl, 2-n-amyl-4-hydroxy methyl-1, 3 dioxolane**)
Hematoxylin**
p,p-Isopropylidenediphenol**
Methyl Salicylate**
Sorbitol*
Styrene Glycol
Thymol "Cellosolve"**
Triethylene Glycol**

V. Recovery of 80 per cent and above

a. *Tested in 10 per cent propylene glycol solution*

d-isoascorbic acid*
2-(Benzyloxy) Ethanol
p-(Benzyloxy) phenol
Benzyl "Carbitol"
1-(2-Biphenylyloxy)-2-Propanol
2,3-Butanediol
2-Butanol,3-(decyloxy)
2-(2-*n*-Butoxyethoxy) ethanol
(2-Butoxyethoxy) Ethanol
Butoxypolypropylene glycol
sec.-Butyl alcohol

2,6-Dimethoxy phenol
2,7-Dimethyl-3,6-Diisopropyloct-4-yne-3,6-diol
10,13-Dimethylocos-11-yne-10,13-diol
2,4-Dimethyl-3-Pentanol
1,1-Diphenyl-2-Methyl Propanediol-1,2
Dipropylene Glycol
Ellagic Acid
9,10-Epoxy octadecanol
2-(2-Ethoxyethoxy) Ethanol
Ethylene Glycol Monobutyl ether
2-Ethyl-1-Hexanol
p-(1-Ethyl-1-methylamyl) phenol

(Table V continued on next page.)

TABLE V—CONT'D

tert.-Butyl alcohol
α-Butyl benzyl alcohol
2-*sec.*-Butylcyclohexanol
n-Butyl lactate
p-A-Cumylphenol
Cyclohexane ethanol
1-(*p*-cyclohexyl phenoxy)-2-Propanol**
p-Cyclohexylxylenol
Cyclopropanemethanol, *α*-methyl
tert.-amylphenol
Diethylene glycol dilactate
Diethylene glycol lactate lactyl lactate
Diethylene Glycol Monomethyl ether
Diethylene Glycolmonolactate
Diethyl-*d*-Tartrate
Diglycerol
Diisobutyl carbinol
Lactic acid, 2-phenoxy ethyl ester
Lauryl Gallate
D (+) Melezitose
2-Methoxyethanol
1-(*α*-Methylbenzyloxy)-2-Propanol
2-Methyl benzyl carbitol
2-Methyl-2-Butenyl Phenol
2-Methyl-2-Furanpropanol
2-Methyl-2,4-Pentanediol
2-Methyl-2-Propyl Propanediol-1,3
β-Naphthol
1,4,7-Octanetriol
2-Octanol
5-Ethylnonanol-2
Geraniol
Glyceryl Mono Stearide
Glycidol
Glycol monolactate
3-Heptanol
1,2-Hexadecanediol
p-hydroxybenzoic acid Methyl salt
1-Hydroxycyclohexane Carboxylic Acid Methyl ester
Hydroxy-Methyl-*p*-*n*-Butylphenol
Inulin
Isoamyl Salicylate
2-Isopropylcyclohexanol
Octyl Gallate
Octyl Hydroquinone
2,2'-Oxydiethanol
Palmitin,2-lacto-1, 3-di
Phenol, *p*-isopentyl
o-Phenylphenate
p-phenylphenol
2,3 dimethyl-2,3-butanediol
1,3-Propanediol
1,2-Propanediol,3-(*p*-nonylphenoxy)
Propylene Glycol Propionate
Pyrocatechol, 4,4'-(2,3-dimethyltetramethylene) di
1,2 Tetradecanediol
2,2,4-Trimethyl-1,3-Pentanediol

b. *Tested in water solution*

Amyl alcohol (*tert.*)**
o-Allyl phenol**
Benzyl alcohol**
Benzyl "Cellosolve"*
1-(4-Biphenylyloxy)-2-propanol**
p,p'-Biphenol**
3-Buten-2-*o*-1,2-methyl
1-Butoxy-2-Propanol
Butyl Cellosolve**
4-*tert.*-Butyl-*o*-cresol**
4-*tert.*-Butylcyclohexanol**
n-Butyl-*p*-Hydroxybenzoate
p,p'-sec.-butylidene diphenol**
p-sec.-Butylphenol**
p-tert.-Butylphenol**
2,2-Diethyl Propanediol-1,3
β, β'-Dihydroxyethyl Ether**
9,10-Dihydroxy-Octadecanol
2,2-Di (*p*-hydroxyphenyl) propane**
3,5-Dimethyl-1-Hexyne-3-*o*-1**
Dimethyl hexynol**
Dimethyl Octynediol**
1,3-diphenoxy propanol*
2,2'-Dipropyl-*p,p'*-Biphenol**
Dipropylene Glycol**
Dipropylene Glycol-*n*-Butyl ether*
Dipropylene glycol methyl ether
Diethylene Glycol Monomethyl ether*
Dipropylene glycol Phenyl ether
2,5-Ditertiary Butyl Hydroquinone**
Ethanol**
2-Ethoxyethanol*
β-Ethoxyethyl lactate**
2-Ethoxymethyl-2,4-dimethyl-1,5-pentanediol*
4-*tert.*-Butyl-2-phenylphenol*
Catechol**
Cinnamyl alcohol**
p-*α*-Cumylphenol**
2-cyclohexylcyclohexanol**
Trans.-1,2-cyclohexanediol**
o-Cyclohexylphenol**
Decamethylene Bisphenol**
5-Decyne-4,7-diol, 2,4,7,9-tetramethyl
2-Desoxy-D-Glucose
2,2'-Diallyl-*p,p'*-bisphenol**
Dicumarol**
Diethylene Glycol Monobutyl ether**
Diethylene Glycol Monoethyl ether*
Glycerol**
Glyceryl Mono Oleate
Guaiacol
1,7-Heptanediol*
1,4,7-Heptanetriol*
2,5-Hexanediol-2,5-Dimethyl*
n-Hexyl alcohol**
n-Hexyl "Carbitol"**
n-Hexyl "Cellosolve"**
Hexylene Glycol**
Hexylresorcinol Soln.**
Hydroquinone**
Hydroquinone Mono-Methyl Ether**
o-Hydroxy Benzyl Alcohol**
Inositol**
Isobutyl alcohol*
2-Isopropoxyethanol*
2-(2-Isopropoxyethoxy) Ethanol*
4,4'-Isopropylidine-di-*o*-cresol**
p-Isopropylphenol**

TABLE V—CONT'D

Ethoxy Triethylene Glycol*	Lactose C. P.**
2-Ethyl Butanol**	Mannite**
2-Ethyl butyl "Cellosolve"**	Mannitol*
Ethylene Glycol**	D (+) Melibiose (hydrate)**
Ethylene Glycol	D (+) Melezitose**
Ethylene glycol monobutyl ether*	Menthol U. S. P.**
Ethylene glycol monoethyl ether*	2-Methoxyethanol*
2-Ethyl-1,3-hexanediol*	2-(2-Methoxy ethoxy) ethanol*
Ethyl Salicylate**	*α*-Methoxy methyl-2,4-dimethyl-1,5-Pentanediol*
Furyfuryl alcohol*	*p*-Phenylphenol**
Galactose**	1-Phenoxy-2-Propanol**
Methoxy Polyethylene Glycol*	Phenyl Salicylate**
3-Methoxy-1,2-Propanediol*	Phloroglucinol**
1-Methoxy-2-Propanol	*dl*-Pinocarveol
Methyl alcohol**	Pinacol**
α-Methyl benzyl alcohol	Polyethylene Glycol 200
2-Methyl benzyl "Cellosolve"*	Polyethylene Glycol 200 Mono Laurate
Methyl butynol**	Polyoxyethylene (20) Sorbitan Monooleate**
Methyl carbinol*	Polyoxyethylene (4) Sorbitan Monolaurate**
Methylene Bis-Para-Cresol	Polyxyethylene (14 Sorbitan Monoleate*
2-Methylglucoside**	Polyoxyethylene (30) Stearate**
Methyl *α*-D Glucoside**	Polyoxyethylene (50) Stearate**
Monoacetin**	Polyoxyethylene (20) Sorbitan Monolaurate**
1-Mono-*n*-butyrin**	Polyoxyethylene (20) Sorbitan Monopalmitate**
1-Monopropionin**	Polyoxyethylene (24) Sorbitan Monostearate*
Morin	Polyoxyethylene Propylene Glycol Monostearate**
1-Myrtenol	Polyoxyethylene (40) Stearate**
β-Naphthol**	Polypropylene Glycol**
Neopentylglycol*	Propylene Glycol (industrial)
Nonyl "Carbitol"**	Polyvinyl alcohol**
1,2-Octanediol**	Propylene Glycol, industrial
3,6-Octanediol-3,6-dimethyl*	Pyrogallol**
1,4,7-Octanetriol*	*d* (+) Raffinose, C.P.*
Octyl Hydroquinone**	l-(-)-Rhamnose*
Orcinol**	Sorbitol*
1,5-Pentanediol	3,3,5-Trimethyl-cyclohexanol**
9,10-Phenanthrenediol, monoacetate	Tripropylene Glycol-*n*-Butyl ether Dowanol 66B*
Phenethyl alcohol*	Tripropylene Glycol methyl ether*
Phenol**	*l-trans.*-Verbenol
2-phenoxyethanol	*dl-trans.*-Verbenol
o-phenylphenate**	*d-trans.*-Verbenol
o-Phenylphenol**	
dl-Sobrerol*	
d-Sobrerol	
l-Sobrerol	
Tetraethylene Glycol**	
Tetrahydrofurfuryl Alcohol*	
1,5-bis-Tetrahydro-2-furyl-3-Pentanol*	
Thymol**	
Triethylene Glycol**	

other phenol derivatives given in Parts I and II of Table V. There as none of these which surpass the inhibitory effect of phenol itself when all substances are tested at 1 per cent or half saturation, whichever is lower. If information on toxicity were available, we might discover that some of the substituted phenols have better therapeutic ratios than does phenol itself.

There are moderately inhibitory substances which deserve special consideration because their toxicity may be low enough to permit them to be considered as food additives for baked goods or confections. Monobutyrin is probably nontoxic since it would be formed in the gastrointestinal tract

during digestion of butter. It is probable that monovalerin, monocaproin and monocaprylin deserve to be studied. The inhibitory effect of some of the lactates and of 1, 2-octanediol are likewise noteworthy because of their potentially low toxicity.

Additional information is required before the practical utility of some of these compounds should be evaluated in clinical testing programs. The toxicity of the more active members of the series should be obtained from the literature or by experimentation. Retest should be made of activity on specially purified samples of compounds to remove the possibility of inhibition by contaminants. Duration of action should be investigated over a period of 12 hours for any substance which is to be considered for use in dentifrice. The most promising substances which remain after this further screening should be tested for their effect on caries of experimental animals.

SUMMARY

Studies have been made on 492 alcohols, phenols, ethers, and esters, in order to determine whether any simple organic chemicals have the capacity to reduce acid formation rate of salivary sediment. A study on monosubstituted ethers and esters or a combination of these 2 groupings indicated that these substances in themselves had no ability to effect acid formation. There were no inhibitory ethers. The one inhibitory ester may have owed its activity to the presence of unesterified alcohol or to the ability of oral enzymes to cause its hydrolysis.

There were a few alcohols or phenols which had the capacity to affect salivary sediment when tested in water solution, but the majority of substances required the presence of 10 per cent propylene glycol for their effect to be demonstrated. In a few instances it appeared that propylene glycol had a synergistic action in with the chemical but in most cases the glycol apparently acted chiefly to increase the solubility of the compound enough to allow it to be inhibitory. Most of the phenols tested at maximum concentration were not superior to phenol itself. A high percentage of inhibitory action occurred among compounds with 6 to 13 carbon atoms, 1 to 3 code units, and ring structures.

We are grateful to the 33 individuals, colleges, or industrial organizations who supplied the alcohols mentioned in Table V, and especially to the Air Reduction Company, Atlas Powder Co., Carbide and Carbon Chemicals Co., Dow Chemical Company, Dr. Harold C. Hodge, Eastern Regional Laboratory, and the Chemical Biological Coordination Center for furnishing us with 10 or more of the compounds. Dr. Paul H. Doleman of Tufts University Chemistry Department has aided us in assigning structures to some of the chemicals.

REFERENCES

1. Manly, R. S.: A Method for the Study of Acid Production by Salivary Sediment, *J. D. Res.* **33**: 561-570, 1954.
2. Manly, R. S., and Hargreaves, G. L.: Inhibition of Acid Production of Salivary Sediments by Various Ethers, Esters, Phenols, and Alcohols, *J. D. Res.* **33**: 672, 1954.
3. Manly, R. S.: Enzyme Inhibitors for Dentifrices, *J. Soc. Cosmetic Chem.* **5**: 266, 1954.
4. Fosdick, L. S., Calandra, J. C., Blackwell, R. Q., and Burrill, J. H.: A New Approach to the Problems of Dental Caries Control, *J. D. Res.* **32**: 486, 1953.
5. Budowski, P., and Markley, K. S.: The Chemical and Physiological Properties of Sesame Oil, *Chem. Rev.* **48**: 125-151, 1951.

SEVERAL PROPERTIES OF SALIVA FROM HUNT-HOPPERT CARIES-RESISTANT AND CARIES-SUSCEPTIBLE RATS

S. ROSEN, M. A. BENARDE, F. W. FABIAN, H. R. HUNT, AND C. A. HOPPERT

Departments of Zoology, Microbiology and Public Health, and Chemistry, Michigan State University, East Lansing, Mich.

THE development of caries-resistant and caries-susceptible strains of rats by Hunt and Hoppert[1-6] has stimulated further microbiologic and physiologic studies to determine the etiology of dental caries in rats.

It is highly probable that microorganisms are the immediate causes of dental caries. The evidence strongly supports the theory that lactobacilli are important factors in the etiology of tooth decay, though other types of bacteria may also be causative agents. It has been shown that lactobacilli are recovered more frequently and in greater numbers from caries-susceptible than from caries-resistant rats.[7-10]

It would seem that the salivas of susceptible and resistant rats might differ physically or chemically and thus account for the higher incidence or potency or cariogenic organisms in the susceptible rats. Also, some specific property of the saliva, if measurable, might enable one to determine the degree of resistance of an animal much sooner than waiting for the appearance of a carious lesion. This would facilitate the study of the inheritance of resistance to caries. Therefore, a study of certain general properties of the salivas was undertaken.

EXPERIMENTAL PROCEDURE

The method of Benarde, Fabian, Rosen, Hunt, and Hoppert[11] was used to collect rat saliva. Caries-susceptible and caries-resistant rats at 4 ages were used, as follows: (1) animals 35 to 55 days. Neither the susceptibles nor the resistants showed caries at this age; (2) animals 80 to 130 days. The susceptibles developed caries during this period, but the resistants did not; (3) animals 180 to 300 days. All of the susceptibles had caries but the resistants had not; and (4) animals over 300 days. Resistant animals which had well-established caries were selected.

In each experiment an equal number of animals was selected from each age group of each strain. The number of determinations depended upon the variability of the results and the availability of animals. When there was a possibility of a significant difference in any one property of the salivas between susceptible and resistant animals, determinations were made using larger numbers of animals from a succeeding generation.

This investigation was supported by a research grant from the National Institute for Dental Research, of the National Institutes of Health, Public Health Service.

Received for publication July 25, 1955.

All tests were performed on untreated saliva except for the specific gravity, surface tension, and relative viscosity determinations. For these determinations, the saliva was filtered through a Seitz pad. Determinations were made on individual rat saliva whenever possible. When pooled salivas were used, they were collected from rats of the same litter. All determinations were begun within one hour after collection of the saliva.

RESULTS

The results are summarized in Table I. The data for all age groups were combined because little or no difference was observed among animals of different ages.

TABLE I
PROPERTIES OF STIMULATED SALIVA OF HUNT-HOPPERT CARIES-RESISTANT AND CARIES-SUSCEPTIBLE RATS

	SUSCEPTIBLE			RESISTANT		
	NUMBER OF DETERMINATIONS	AVERAGE	RANGE	NUMBER OF DETERMINATIONS	AVERAGE	RANGE
pH	10	8.35	8.00-8.70	7	8.18	7.96-8.44
Amylase—trial 1	4	29.99 mm.	27.0-33.2 mm.	4	26.54 mm.	24.3-29.5 mm.
Amylase—trial 2	30	24.21 mm.	16.0-28.0 mm.	31	23.76 mm.	15.0-27.0 mm.
Relative viscosity—trial 1	4	1.09	1.06-1.11	4	1.19	1.17-1.20
Relative viscosity—trial 2	32	1.27 ± .03	1.02-1.67	32	1.42 ± 0.4	1.17-2.14
Relative viscosity—trial 3	33	1.55 ± .04	1.10-1.86	20	1.67 ± .04	1.38-2.08
Surface tension	11	43.91 dynes/cm.	42.2-46.0 dynes/cm.	10	42.94 dynes/cm.	41.9-44.3 dynes/cm.
Refractive index	12	1.3348	1.3339-1.3361	12	1.3344	1.3339-1.3358
Specific gravity	11	0.9990	0.9948-1.0008	11	1.0005	1.0001-1.0008
Agglutinin titer for rat oral lactobacilli	33	— —	0-1:256	31	——	0-1:256
Antagonistic effect against rat oral lactobacilli	24	0.0 mm.	——	24	0.0 mm.	——

Determinations of pH were made electrometrically using the conventional glass electrode within one-half hour after collection of the saliva. No differences were found; salivas from both strains were definitely alkaline. Since it was possible that the pH of saliva might have changed before analysis, the

pH was determined in the mouths of the animals by inserting a strip of Fisher alkacid paper. The pH was approximately 8.0 for 10 susceptible and 10 resistant animals.

Determinations for *amylase* were made by the method of Stark, Wellerson, Tetrault, and Kossack.[12] Filter paper discs are placed on starch agar gels and impregnated with 0.07 ml. of saliva. After 10 hours' incubation, the surface of the starch agar gel is flooded with iodine and the diameters of the colorless zones measured against a blue background. The average zones of hydrolysis were approximately the same for both susceptible and resistant animals. No attempts were made to determine the exact amount of amylase present, but a 0.1 per cent alpha amylase solution produced a zone with a diameter measuring 31.0 to 34.0 mm.

Viscosity determinations were made in a 1 ml. volumetric pipette which was constricted by heating so that distilled water, falling between 2 points, had a dropping time of at least 15 seconds. The dropping time of saliva was related to the dropping time of distilled water at room temperature. This property showed the most promise of distinguishing the salivas from resistant and susceptible rats. In 3 trials the relative viscosity of saliva from resistant rats was found to be 9, 11, and 7 per cent higher than that of susceptible rats.

In trial 2, the viscosity of the saliva from resistant rats exceeded that of the susceptibles by 0.15 ± 0.05. The t value is 3.0, which is significant at the 0.3 per cent level. The viscosity of the saliva from the resistants in trial 3 exceeded the viscosity for the susceptibles by 0.12 ± 0.06. The t value is 2.0, which is significant at about the 5 per cent level.

The mean viscosity of the saliva from susceptibles increased by 0.28 ± 0.05 from the second to the third trial. The t value is 5.6, so that the difference is highly significant, at least at the 0.0002 per cent level. A similar increase occurred for the resistants. There was an increase of $0.25 \pm .06$ which gives a t value of 4.2, so that the differences between successive trials within the same strain were greater than the differences between strains. The difference in successive trials may have been influenced by season of year, since trial 2 was carried out in the fall and trial 3 in the winter. Another explanation could be that each trial represented animals from a succeeding generation.

Although there is considerable difference in relative viscosity between trials and the distribution of viscosities of susceptibles and resistants overlap extensively, there is still reason to believe that the saliva of resistant rats may be more viscous than that of susceptibles since the average viscosities were higher for saliva from resistant animals in each of the 3 trials.

The *surface tension* of saliva was measured by a torsion balance method in which a Cenco Du Nouy interfacial tensiometer was employed. Average values of 43.91 dynes/cm. for susceptibles and 42.94 dynes/cm. for resistants were obtained.

An AO Spencer refractometer (Series 424) was used to determine the *refractive index* of saliva. The average value for the susceptibles was 1.3348 and for the resistants 1.3344.

Determinations of *specific gravity* were made with the aid of a 1.0 ml. Lipkin-type pycnometer. The average value for the susceptibles was 0.9990 and for the resistants 1.0005. Too much emphasis should not be placed on this difference since 8 out of the 11 susceptibles tested had an average specific gravity of 1.0005, the same as the average for the resistant animals.

Certain references in the literature suggested that lower oral Lactobacillus counts or decreased caries activity might be correlated with a high *agglutinin titer* for lactobacilli in the saliva.[13-16] Tube agglutination tests were performed on saliva using 4 strains of oral lactobacilli encountered most frequently in the Hunt-Hoppert caries-susceptible rats. Titers ranged from 0 to 1.256 for both susceptible and resistant rats. The titers tended to be higher in the resistant strain, but the results were highly variable.

Several investigators have reported that saliva is able to inhibit certain strains of lactobacilli.[17-21] Therefore, it was possible that saliva from resistant rats might *inhibit rat oral lactobacilli* whereas that from susceptible animals might not. Rather than use individuals strains of rat oral lactobacilli, swabs were made of the teeth of susceptible animals and streaked on the surface of LBS medium (BBL) and tomato juice agar special medium (Difco). Enough material was placed on the above agars so that approximately one thousand colonies appeared. Immediately after the plates were swabbed, deep wells were cut, and 0.3 ml. of saliva was used to fill each well. In some cases the plates were immediately incubated, but in other instances they were refrigerated for 24 to 48 hours before incubation. Twenty-four tests made with each type of saliva failed to show any inhibition whatsoever in the growth of lactobacilli.

DISCUSSION

The wide difference in caries behavior of the Hunt-Hoppert caries-resistant and caries-susceptible rats suggests 1 or more differences between the 2 strains that are significant and consistent.

Of the 8 properties of the saliva studied, 7 showed no significant difference between the susceptible and resistant strains. There may be a real difference in viscosity, and this question merits further investigation.

Investigations on several of the other properties might be extended. For example, in addition to tube agglutination tests, other immunologic studies such as precipitin and complement fixation tests might be used to detect differences between susceptible and resistant rats. Also, other strains of rat oral lactobacilli might be used as test antigens. Studies on the inhibitory effect of saliva could be broadened to include individual strains of rat oral lactobacilli and even streptococci.

Possibly, the most significant data were obtained from the studies on the viscosity of saliva. Although the results were rather variable and there was an unexplainable increase in the relative viscosity of the later generations of rats, there is a slight tendency for the saliva from resistant animals to be more viscous. This indicates that saliva from susceptible animals may contain enzymes in greater quantity than resistant animals which are capable of degrading large molecules. The breakdown products could be utilized by cariogenic bacteria.

One must consider that the results obtained in this study were with stimulated saliva which undoubtedly contains fluids secreted from the lungs, bronchi, nose, and mucosa of the mouth. It is also possible that pilocarpine-stimulated saliva may be different from the saliva as it is normally found in the rat.

SUMMARY

The following properties of the saliva of the Hunt-Hoppert caries-resistant and caries-susceptible rats have been studied: pH, amylase activity, relative viscosity, surface tension, refractive index, specific gravity, agglutinin titer for rat oral lactobacilli, and antagonistic effect of saliva against rat oral lactobacilli. The relative viscosity tended to be slightly, and possibly significantly higher in saliva from resistant rats. In the case of all of the other properties studied, no differences were found.

REFERENCES

1. Hunt, H. R., and Hoppert, C. A.: Inheritance in Rat Caries, *Genetics* **24**: 76, 1939.
2. *Idem:* Inheritance in Rat Caries, Year Book of the American Philosophical Society, 1941, p. 131.
3. *Idem:* Inheritance of Susceptibility to Caries in Albino Rats (Mus norvegicus), *J. Am. Col. Dentists* **11**: 33, 1944.
4. Hunt, H. R., Hoppert, C. A., and Braunschneider, G. E.: Heredity and Environmental Factors Influencing Tooth Decay in the Albino Rat (Rattus norvegicus), *Records of the Genetics Society of America* **16**: 37, 1947.
5. Hunt, H. R., Hoppert, C. A., and Erwin, W. G.: Inheritance of Susceptibility to Caries in Albino Rats (Mus norvegicus), *J. D. Res.* **23**: 385, 1944.
6. Hunt, H. R., and Hoppert, C. A.: Occlusion as a Factor in Dental Caries of Albino Rats (Rattus norvegicus), *J. D. Res.* **27**: 553, 1948.
7. Jay, P., Hunt, H. R., and Hoppert, C. A.: The Incidence of Oral *L. acidophilus* in Hunt-Hoppert Caries Resistant and Caries Susceptible Rats (Abstr.), *J. D. Res.* **23**: 205, 1944.
8. Jay, P.: The Effect of Substrate on the Oral Flora, *J. A. D. A.* **37**: 416, 1948.
9. Rosen, S., Benarde, M. A., Hunt, H. R., and Hoppert, C. A.: Microbiologic Differences in the Oral Cavities of the Hunt-Hoppert Caries-Resistant and Caries-Susceptible Rats, *J. D. Res.* **34**: 113, 1955.
10. Rosen, S., Hunt, H. R., and Hoppert, C. A.: The Frequencies of Oral Lactobacilli in the Hunt-Hoppert Caries-Resistant and Caries-Susceptible Rats in Relation to the Development of Caries, *J. D. Res.* **35**: 586, 1956.
11. Benarde, M. A., Fabian, F. W., Rosen, S., Hoppert, C. A., and Hunt, H. R.: A Method for the Collection of Large Quantities of Rat Saliva, *J. D. Res.* **35**: 326, 1956.
12. Stark, E., Wellerson, R., Jr., Tetrault, P. A., and Kossack, C. F.: Bacterial Alpha Amylase Paper Disc Tests on Starch Agar, *Appl. Microbiol.* **1**: 236, 1953.
13. Jay, P., Crowley, M. C., and Bunting, R. C.: Relationship and Occurrence of Bacillus acidophilus in Humans to Skin Sensitivity and Specific Agglutinins in Blood Serum, *J. D. Res.* **12**: 429, 1932.
14. Jay, P., Crowley, M. C., and Bunting, R. C.: Preliminary Studies on the Immunology of Dental Caries, *J. A. D. A.* **19**: 265, 1932.
15. Canby, C. P., and Bernier, J. L.: Bacteriologic and Immunologic Studies in Dental Caries: A Preliminary Report, *J. A. D. A.* **29**: 606, 1942.
16. Dietz, V. H., Williams, N. B., and Lawton, W. E.: Relationships Between Blood Agglutinins for Oral Lactobacilli, Salivary Counts and Caries, *J. A. D. A.* **30**: 838, 1943.
17. Clough, O. W.: Inhibition of Bacterial Growth by Human Saliva, *J. D. Res.* **14**: 164, 1934.
18. *Idem:* Inhibitory Effects of Saliva on Lactobacillus acidophilus, *J. D. Res.* **15**: 213, 1935.
19. Hine, M. K.: Daily Observations of Bacterial Inhibition by Saliva, *J. D. Res.* **15**: 305, 1936.
20. Bibby, B. G., Clough, O. W., and Hine, M. K.: The Antibacterial Action of Human Saliva, *J. A. D. A.* **25**: 1290, 1938.
21. Hill, T. J.: A Salivary Factor Which Influences the Growth of Lactobacillus acidophilus and Is an Expression of Susceptibility or Resistance to Dental Caries, *J. A. D. A.* **26**: 239, 1939.

DECREASED VASCULARITY OF THE DENTAL PULP

I. The Effects of Unilateral Ligation of Major Vessels to the Teeth and of Combined Ligation-Salivary Adenectomy on Dental Caries Experience in the Rat

HAROLD E. BREWER, JOSEPH C. MUHLER, AND ROBERT B. FISCHER

Departments of Anatomy and Chemistry, Indiana University, Bloomington, Ind.

IT HAS previously been reported that bilateral ligation of the major blood vessels supplying the molar teeth of rats results in a significant increase in the incidence of dental caries.[1] To further delineate the effect of ligation it was considered important to compare the bilateral incidence of dental caries in animals having the blood vessels to the molar teeth ligated unilaterally. Furthermore, since the possibility existed that ligation of the major blood vessels supplying the molar teeth altered the function of the salivary glands by affecting the blood flow to the glands, additional work was performed in order to study the effect of combined ligation-salivary adenectomy to salivary adenectomy alone. Such a comparison should more clearly indicate the relative effects of each operation and help to clarify the mechanism by which ligation of the vessels increases caries.

EXPERIMENTAL

In Series I of these studies, unilateral ligations of the right internal and external maxillary arteries were performed in a group of 30 weanling Sprague-Dawley strain rats. The surgical technique employed was identical to that previously reported in detail.[1] In Series II, a total of 120 weanling Sprague-Dawley rats were divided equally into 4 groups. Unilateral ligations of the right internal maxillary artery were performed on the animals in Group 1. Salivary adenectomies, i.e., removal of the submandibular, sublingual, and ventrolateral portions of the parotid glands, were performed bilaterally on the animals comprising Group 2. In Group 3, the operations for ligation and salivary adenectomy were combined so that each animal in the group had the major blood vessels to the right molar teeth ligated and, in addition, had the major salivary glands removed bilaterally. The fourth group was unoperated and served as controls. In both series, the animals were housed in pairs in metal cages and received a cariogenic stock corn diet (F — 0.5 ppm) and tap-distilled water (F — 0.05 ppm) ad libitum throughout the experimental period of 140 days.[2] At the termination of the experimental period, the animals were sacrificed by chloroform and the heads removed for dental caries evaluation as reported previously.[3]

This study was supported in part by a grant from the United States Public Health Service.

Received for publication July 29, 1955.

DATA AND DISCUSSION

A summary of the data obtained from the study in which the right internal and external maxillary arteries were ligated is shown in Table I (Series I). There is a significant increase in the incidence of dental caries in both the right maxillary and mandibular molar teeth. These data corroborate the previous findings which also indicated an increase in the incidence of dental caries when the ligation operation was performed bilaterally and compared to unoperated controls.[1]

TABLE I
A COMPARISON OF THE MEAN NUMBER OF CARIOUS LESIONS IN RATS HAVING AN ALTERED BLOOD SUPPLY TO THE TEETH ON THE RIGHT SIDE WITH THE LEFT SIDE SERVING AS THE CONTROL (SERIES I)

DENTAL ARCH		DENTAL CARIES INCREMENT (MEAN NO. OF LESIONS)		
		MAXILLARY MOLARS	MANDIBULAR MOLARS	HALF-MOUTH TOTAL
Right		1.36	4.52	5.4
Left		0.64	2.15	3.2
	"t"	2.32	6.10	4.86
	P	>0.02	0.0001	>0.001
	% Increase	102	110	69

A comparison of the dental caries experience of the animals with ligated blood vessels and those with the major salivary glands removed is seen in Table II (Series II). Columns 5 and 6 in Table II indicate the incidence of dental caries in the left maxillary and mandibular molars. In none of these animals was the ligation operation performed. It is interesting to note that salivary adenectomy does not significantly increase the incidence of dental caries in the maxillary teeth but does have a pronounced effect in the mandibular arch. These findings corroborate previous observations from this laboratory. The reason for this is unknown, but additional studies are needed to explain the phenomenon. However, when the data comprising columns 7 and 8 are examined, in which the ligation operation to the right molar teeth

TABLE II
A COMPARISON OF THE MEAN NUMBER OF CARIOUS LESIONS IN RATS HAVING THE RIGHT INTERNAL MAXILLARY ARTERY LIGATED; IN BILATERALLY SALIVARY ADENECTOMIZED; IN CONCOMITANTLY LIGATED-SALIVARY ADENECTOMIZED; AND IN CONTROL RATS (SERIES II)

GROUP	NO. OF ANIMALS	SEX	MEAN TOTAL BODY WEIGHT (GM.)	DENTAL CARIES INCREMENT (MEAN NO. OF LESIONS)					
				LEFT MOLARS		RIGHT MOLARS		HALF-MOUTH TOTALS	
				MAX.	MAND.	MAX.	MAND.	LEFT	RIGHT
Control	26	M F	364 232	0.38	1.73	0.35	1.92	2.12	2.27
Ligated	18	M F	371 241	0.61	1.29	1.00*	3.79*	1.89	4.78*
Salivary adenectomized	12	M F	254 202	0.58	4.59*	0.42	4.66*	5.17*	5.08*
Ligated and salivary adenectomized	11	M F	280 214	0.18	4.64*	4.27*	5.45*	4.92*	9.73*

*Indicates a difference from corresponding control at 0.01 confidence level or greater.

was performed, a distinct difference in the dental caries increment is noted. There is a significant increase in dental caries in the ligated animals in the maxillary arch but again not in the salivary adenectomized rats. The difference is much greater when the combined ligation-salivary adenectomy is performed, as evidenced by the greater magnitude of dental caries in this group as compared to the incidence in the maxillary molars on the left side of the arch.

Similarly in the mandibular arch (column 8) the cariogenic effect of the ligation operation is evident for there is found a significant increase in the ligated animals when compared to the unoperated controls. In the mandibular arch there is a significant increase in the incidence of dental caries in the combined ligation-salivary adenectomized rats but the difference when compared to the same operations in the maxillary arch is not as great. These data would seem to indicate that the ligation operation has a significant effect in increasing dental caries in the maxillary arch and since this is not found in salivary adenectomized rats, the cariogenic mechanism of the 2 operations must be distinctly different.

The effect of the ligation operation on the final body weight of the animals is quite interesting. Previous workers have reported that salivary adenectomized rats[4, 5] and hamsters[6, 7] fail to gain as much weight as unoperated rats. The data in this experiment corroborate these data. Furthermore, additional evidence that the effects of ligation are distinct from those of salivary adenectomy is indicated by the final weight of the ligated animals, for it is seen that no body weight loss is found in the ligated groups. In the ligated groups there is a small increase in weight. This is evident further when comparing the ligation-salivary adenectomy group with the salivary adenectomy group alone.

SUMMARY AND CONCLUSIONS

The data permit the following conclusions:

1. Experimental interruption of the vascular supply to the molar teeth results in increased dental caries in both maxillary and mandibular teeth, while salivary adenectomy increases dental decay only in the mandibular molars.

2. A combination of the 2 operations in the same rat results in increased dental caries incidence to a greater degree than each operation results in alone.

REFERENCES

1. Brewer, H. E., Muhler, J. C., and Fischer, R. B.: Decreased Vascularity of the Dental Pulp. I. Its Effects on Dental Caries Experience in the Rat, *J. D. Res.* **34**: 674, 1955. (Abst.)
2. Muhler, J. C., and Day, H. G.: Effects of Stannous Fluoride, Stannous Chloride and Sodium Flouride on the Incidence of Dental Lesions in Rats Fed a Caries Producing Diet, *J. A. D. A.* **41**: 528, 1950.
3. Muhler, J. C., Nebergall, W. H., and Day, H. G.: Preparations of Stannous Fluoride for the Prevention of Dental Caries in the Rat, *J. A. D. A.* **46**: 290, 1953.
4. Bixler, D., Muhler, J. C., and Shafer, W. G.: Experimental Dental Caries. IV. The Effects of Desalivation and Castration on Caries Incidence and Fluorine Storage in the Rat, *J. Nutrition* **52**: 345, 1954.
5. Muhler, J. C., and Shafer, W. G.: Experimental Dental Caries. II. Effect of Desalivation on Dental Caries and Castration and Desalivation Fluoride Storage in the Rat, *J. D. Res.* **33**: 346, 1954.
6. Gilda, J. E., and Keyes, P. H.: Increased Dental Caries Activity in the Syrian Hamster Following Desalivation, *Proc. Soc. Exper. Biol. & Med.* **66**: 28, 1947.
7. Dale, P. P.: Caries Studies in Desalivated Rats Receiving Iodoacetic Acid Parenterally, *J. D. Res.* **27**: 757, 1948.

EXPERIMENTAL DENTAL CARIES

IX. Relationship of Thyroid Function to Dental Caries Experience in Salivary-Adenectomized Rats

JOSEPH C. MUHLER AND WILLIAM G. SHAFER

From Indiana University, Department of Chemistry and School of Dentistry, Bloomington and Indianapolis, Ind.

PREVIOUS work has clearly shown that a relationship exists between the activity of the thyroid gland and the incidence of experimental dental caries in the rat. For example, desiccated thyroid added to the diet of rats significantly reduces the dental caries experience while thiouracil significantly increases their dental caries.[1] Furthermore, when thyroid function was destroyed by the administration of I^{131} a similar increase in rat dental caries was found as was noted with the administration of thiouracil.[2] The mechanism by which the thyroid gland functions in these processes is not known, but it is thought that the salivary glands are involved in some intermediate manner. Indirect support for this conclusion is found in the observation that desiccated thyroid-testosterone administration reduces the incidence of rat dental caries to a greater degree than desiccated thyroid alone.[3] This observation is in keeping with previous work which indicates that testosterone increases the activity of the thyroid gland,[4] and that the thyroid hormone is necessary for the normal physiology of the salivary glands.[5] It was felt, however, that more direct evidence might be secured if the function of the thyroid gland is studied in the absence of the major salivary glands. The purpose of this investigation, then, is to study the effect of desiccated thyroid and thiouracil administration in salivary-adenectomized animals. The results should more clearly indicate the mechanism by which the thyroid gland is related to the dental caries experience in rats.

PROCEDURE

This study is divided into 2 parts, Series 1 and Series 2. The only difference between the 2 was that Series 1 was started approximately three months prior to Series 2. The reason for repeating the experiment with identical groups was due to the high mortality in the salivary-adenectomized groups receiving thyroid and testosterone. It was considered of importance to repeat the study in an attempt to increase the numbers of animals finishing the study. However, in effect, if both experiments corroborate each other the procedure would serve to strengthen the conclusions of the study as a whole.

This investigation was supported in part by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-332.

Received for publication Aug. 18, 1955.

TABLE I
COMPARISON OF DENTAL CARIES INCIDENCE IN DESALIVATED RATS RECEIVING DESICCATED THYROID, THIOURACIL, OR TESTOSTERONE

GROUP	SERIES 1					SERIES 2				
	NO. OF RATS	FINAL WT. (GM.)	NO. OF LESIONS	% INCREASE COMP. TO CONTROLS	% INCREASE COMP. TO DESALIVATES	NO. OF RATS	FINAL WT. (GM.)	NO. OF LESIONS	% INCREASE COMP. TO CONTROLS	% INCREASE COMP. TO DESALIVATES
Control	15	241	4.7	—	–33	21	192	4.3	—	–41
D*	15	154	7.0	+50	—	19	168	7.3	+70	—
D + T	15	169	8.0	+70	+14	17	164	8 9	+107	+16
D + Th	16	114	10.1	+113	+30	14	107	10.7	+133	+47
D + Thy	15	163	8.3	+77	+19	14	138	8.1	+88	+11
D + Th + T	14	103	10.9	+124	+41	21	106	11.6	+141	+59
D + Thy + T	12	159	7.8	+66	+11	14	150	9.1	+110	+25

*D = Desalivation; T = Testosterone; Th = Thiouracil; Thy = Thyroid.

In both Series 1 and 2, 7 groups of male weanling rats were divided equally according to initial body weight. Group 1 served as a control, and was neither salivary-adenectomized nor supplemented with the thyroid preparations. Group 2 animals had their major salivary glands removed between the ages of 27 and 30 days, according to methods previously described.[6] Group 3 was similarly salivary-adenectomized and in addition received an aqueous suspension of 1.25 mg. of testosterone per week per animal injected intramuscularly. A fourth group was salivary-adenectomized and in addition received propylthiouracil (0.1 per cent) in the diet. Group 5 was salivary-adenectomized and received desiccated thyroid in the diet (in increasing amounts from 5 to 15 mg. per day calculated by food consumption and depending upon the growth of the animal). Groups 6 and 7 were both salivary-adenectomized and each received testosterone at the same level as the animals in Group 3, but in addition, Group 6 received thiouracil and Group 7 received desiccated thyroid, each supplement being furnished in the diets at the same level as given to animals in Groups 4 and 5 respectively.

The duration of the experiment was 77 days in Series 1, and 89 days in Series 2. All of the animals were housed in raised screen cages and received the same cariogenic stock corn diet (fluorine — 0.5 mcg. per gram) and fluorine-low (F — 0.5 mcg. per milliliter) drinking water. At the termination of the experimental period, the animals were sacrificed by chloroform and the heads removed for dental caries evaluation by methods previously described.[7]

RESULTS

The results of these experiments are seen in Table I. The incidence of dental caries in the desalivated animals of series is significantly different from the control groups at the 0.01 level of confidence. When the groups are compared to the salivary-adenectomized groups, the control, the desalivate-thiouracil, desalivate-thiouracil-testosterone groups in both Series 1 and 2 are significantly different at the 0.01 level. None of the other groups in either series are significantly different when compared to the salivary-adenectomized groups except the desalivate-thyroid-testosterone group in Series 2, which is significant at the 0.05 level.

DISCUSSION

In both series salivary adenectomy increases the incidence of dental caries. These data confirm much previous work,[6, 8] and clearly indicate the effect on dental caries of the absence of normal salivary gland function. It should be noted that the amount of dental caries in both the control and salivary-adenectomized groups is clearly less than that reported by these laboratories previously. The reason for this is that in all former experiments the duration of the experimental period was from 125 to 140 days, while in this study the mortality of the desalivate-thyroid-testosterone groups limited the duration to approximately 80 days.

These data show in a convincing manner that the pronounced anticariogenic effect of desiccated thyroid is promptly lost in the absence of the major

salivary glands. The cariogenic effect of thiouracil is still evident, but appears to be less intense in the salivary-adenectomized animals than when the thiouracil is administered to animals with intact salivary glands.[3] These data would seem to implicate the major salivary glands as playing a major role in the thyroid-dental caries interrelationship. Furthermore, testosterone seems to augment the effect of thiouracil instead of resulting in an anticariogenic effect as occurs with desiccated thyroid. In fact, in both series, the desalivate-thiouracil-testosterone groups have the greatest incidence of caries that is found in any other group. Furthermore, these data would indicate that the results of previous studies in which desiccated thyroid was found to reduce the dental caries experience is not a result of any topical action of the compound with the erupted teeth, but is due instead to some effect on the salivary glands.

The role of the minor salivary glands needs to be considered in such studies in explaining the mechanism of action. It would seem that if the effect of thiouracil were to diminish the flow of saliva directly, it would also affect the minor salivary glands comparably. This might be indicated in the sum of effects shown by the results of the desalivate-thiouracil group. However, this would suggest that the desalivate-thyroid group might show a reduction in caries when compared to the salivary-adenectomized group if there is an increase in function in the minor salivary glands. Since in neither series did the desalivate-thyroid group show a reduction in dental caries when campared to the salivary-adenectomized group, one must conclude that either the minor salivary glands are not directly involved or else the conditions under which they may compensate for the major salivary gland loss have not been met in these studies. Further work is needed to clarify not only the role of the minor salivary glands, but also to indicate if salivary function (flow, viscosity, etc.) is modified as a result of hyper- or hypothyrodism.

The procedure of salivary adenectomy to which the animals were subjected appears to have marked effects on their general well-being. Previous work in these laboratories has indicated that the removal of the salivary glands caused a marked decrease in weight gain.[6, 8] This effect was not due entirely to inanition, for when control rats were paired-fed to salivary-adenectomized rats the males still failed to gain as much weight while eating identical amounts of food.[6] Furthermore, in previous studies desalivation has been clearly shown to cause a generalized atrophy of the uterus.[8] These effects also have been shown to persist in salivary-adenectomized rats and not in control rats which were restricted to the food consumption of the desalivated rats.[9] These and similar studies indicate that more pointed information regarding the nutrition of the salivary-adenectomized rat, the pH of the oral contents, bacterial flora of the mouth, etc., is needed.

SUMMARY

The anticariogenic effectiveness of desiccated thyroid is not evident when this material is fed in the diet to rats with the major salivary glands removed. Similarly, the cariogenic effectiveness of thiouracil, while still manifested in salivary-adenectomized animals, seems to be reduced in comparison to the

cariogenic effect of this compound when fed to rats with intact salivary glands. These observations lend credence to the hypothesis that the salivary glands are directly involved in the thyroid-dental caries relationship.

REFERENCES

1. Muhler, J. C., and Shafer, W. G.: Experimental Dental Caries, IV. The Effect of Feeding Desiccated Thyroid and Thiouracil on Dental Caries in Rats, *Science* **119**: 687, 1954.
2. Bixler, D., Muhler, J. C., and Shafer, W. G.: The Effect of Radioactive Iodine on Dental Caries in the Rat, *J. A. D. A.* **53**: 667, 1956.
3. Muhler, J. C., and Shafer, W. G.: Experimental Dental Caries, VIII. Effect of Thyroid-Testosterone Administration on Dental Caries in Rat, *Proc. Soc. Exper. Biol. & Med.* **88**: 191, 1955.
4. Burris, M. J., Bogart, R., and Kruegar, H.: Alteration of Activity of Thyroid Gland of Beef Cattle With Testosterone, *Proc. Soc. Exper. Biol. & Med.* **84**: 181, 1953.
5. Shafer, W. G., and Muhler, J. C.: The Effect of Desiccated Thyroid, Propylthiouracil, Testosterone and Fluorine on the Submaxillary Glands of the Rat, *J. D. Res.* **35**: 922, 1956.
6. Bixler, D., Muhler, J. C., and Shafer, W. G.: Experimental Dental Caries, V. The Effects of Desalivation and Castration on Caries and Fluorine Storage in the Rat, *J. Nutrition* **52**: 345, 1954.
7. Muhler, J. C., Nebergall, W. H., and Day, H. G.: Comparison of Different Preparations of Stannous Fluoride in the Prevention of Dental Caries in the Rat, *J. A. D. A.* **46**: 290, 1953.
8. Muhler, J. C., and Shafer, W. G.: Experimental Dental Caries, II. Effect of desalivation on Dental Caries and Castration and Desalivation on Fluorine Storage in the Rat, *J. D. Res.* **33**: 346, 1954.
9. Bixler, D., Muhler, J. C., and Shafer, W. G.: Effect of Desalivation on Adrenals, Uterus, and Testes in the Rat, *J. D. Res.* **34**: 910, 1955.

BIOCHEMICAL ANALYSIS OF HUMAN SALIVARY MUCIN

I. The Possibility of Cholesterol in Mucin

M. IRWIN AND A. G. LEAVER

Medical Research Council Department, University of Otago Dental School, Dunedin, New Zealand

WORK in this laboratory is at present concerned with biochemical analysis of human salivary mucin. This paper is introductory, in that the methods of obtaining mucin are critically discussed, but includes an investigation of the possibility of lipid materials being an integral part of the mucin complex, as reported by Kazakova.[1]

THE PREPARATION OF HUMAN SALIVARY MUCIN

The collection and pooling of overnight samples of saliva was highly unsatisfactory. Such saliva contained large masses of bacteria and debris and was usually a watery secretion of low mucin content. The ideal method is for one subject to spit directly into the appropriate precipitating solution over a long period of time. In this way the possibility of enzymic depolymerization taking place, as described by Knox and Still,[2-4] is avoided. In view of the large volume of saliva required, this method was considered impracticable.

It was finally decided to fit traps into the circuits of the saliva ejectors in one of the clinics of the Dental Hospital. Twenty bottles, of about 250 ml. capacity each, were used in this way; being emptied immediately after the patient had left the chair. The saliva was then placed in the refrigerator for a short time and removed for centrifuging. Saliva collected in this way was usually thick and relatively viscous. After centrifuging for 30 minutes at 2,300 r.p.m., the amount of bacteria, debris, etc., was very small by comparison with that obtained from "resting" saliva. Whenever saliva was to be used for mucin precipitation, it was always subjected to this preliminary centrifuging.

Clement[5] mentioned various technics for mucin precipitation most of which involve acidification, often in conjunction with addition of alcohol, acetone, etc. Other technics involve the use of specific precipitants such as phosphotungstic or trichloracetic acids.

According to Inouye,[6] the isoelectric point of mucin is between pH 2.75 to 2.95, although work in this laboratory suggests that it may be slightly further to the acid side. On adding 1 per cent acetic acid to saliva, some minor degree of precipitation was observed at pH 5.0, which precipitate seem to redissolve

Received for publication June 15, 1955.

at lower pH. Thus, 2.3 mg. of material was obtained from 1 liter of centrifuged saliva. It appeared to be protein but was obviously not associated with the mucin complex. This was not pursued further.

Addition of an equal volume of 1 per cent acetic acid to saliva produced a pH of 3.9 while the addition of 2 volumes reduced the pH to 3.55. It was therefore decided to add glacial acetic acid until the pH was reduced to 2.65. In practice this involved the addition of between 12 to 16 per cent of the volume of saliva being treated. A volume of acetone, equal to the combined saliva and acetic acid volume, was then added and the mixture allowed to stand overnight in the refrigerator. The mucin settled out and, after decanting off the liquid, the precipitated material was washed with acetone and dried in air at 37° C. The amount of dry mucin obtained in this way has varied from 0.20 to 0.41 Gm. per liter of saliva. Figures quoted in the literature vary enormously with different methods of precipitation and even when similar methods are used.

It is appreciated that our method of precipitation followed by air drying is drastic and would be entirely unsuitable in studies of the physical properties of mucin. It is, however, suitable for analytical work and provides a simple method of obtaining a relatively large amount of the dry mucin for this purpose.

EXAMINATION OF MUCIN FOR LIPID COMPONENTS

Kazakova[1] precipitated mucin from centrifuged human and canine saliva by the use of 1 per cent acetic acid, followed by reprecipitation with water after taking up the original precipitate in 0.1N HCl. Mucin was extracted with boiling ethanol for 6 hours. The mucin residue after filtration was washed with ether, the washings being combined with the alcohol extracts and the whole evaporated to dryness. Qualitative tests indicated the presence of cholesterol which was later quantitatively determined. A cholesterol concentration of 1 to 1.5 Gm. per 100 Gm. of human salivary mucin was reported, the value for canine mucin being 0.5 to 0.62 Gm. per 100 Gm.

Kazakova concluded that as cholesterol cannot be removed by washing the mucin with ether, but only by boiling with ethanol, it is an integral part of the mucin complex which is therefore considered to be a "gluco-lipo-protein."

EXPERIMENTAL METHODS AND RESULTS

Only a minor degree of precipitation of mucin was achieved by the use of 1 per cent acetic acid and it was decided to use the technic previously described for the precipitation of mucin.

Mucin, precipitated in this way, was well washed with aqueous acetone to remove all traces of acetic acid before being dried in air and weighed. Four experiments, using from 0.33 to 2.43 Gm. of dried mucin, were performed. As the results were qualitatively the same in all four cases, the full quantitative details of only one experiment are given.

In this experiment, 2.43 Gm. of the dried mucin were refluxed with absolute ethanol for 6 hours. The ethanol was filtered off and the mucin residues washed

with ether, the washings being added to the ethanol filtrate and the whole evaporated to dryness under reduced pressure. The weight of material removed in this way was 16 mg. The material was warmed with 60 per cent aqueous KOH (10 ml.) and allowed to stand under nitrogen for 16 hours. After dilution with water, ether extraction of the aqueous solution removed 6 mg. of material which may be regarded as the unsaponifiable fraction. This was taken up in 5 ml. of warm 95 per cent ethanol and added to an excess of 0.4 per cent digitonin in 95 per cent ethanol. After standing overnight the minute amount of precipitate was centrifuged down and weighed. From this weight (4 mg.), it was calculated that there was present in the unsaponifiable fraction, less than 1 mg. of a sterol. The Liebermann-Burchard test gave a faint pink color but no suggestion of the characteristic blue color associated with the presence of cholesterol.

In another experiment, using 2.13 Gm. of salivary mucin, the unsaponifiable matter amounted to 5 mg. and gave only the faintest opalescence with digitonin, no precipitate being recoverable. The Liebermann-Burchard test was negative. The ultraviolet absorption of the unsaponifiable fraction in methanol (A. R.) exhibited a broad peak between 275 to 285 mμ. The absorption spectrum of both ergosterol and 7-dehydrocholesterol is given by Morton.[7] They exhibit main maxima at 281 and 271 mμ with a small peak at 293.5 mμ.

A pink color in the Liebermann-Burchard test is associated with 7-dehydrocholesterol and this, together with the spectrum, might suggest the presence of a minute trace of this sterol.

It was finally decided to attempt a recovery experiment in which 20 mg. of cholesterol in alcohol was added to 1 liter of centrifuged saliva. The mucin precipitated from this saliva was extracted with chloroform and the extracts examined for sterol. Only 4.7 mg. of sterol, which gave a distinctly positive Liebermann-Burchard reaction, was recovered.

The chloroform-washed mucin (817 mg.) was subjected to the usual ethanol reflux procedure. On taking the ethanol solution to dryness, the amount of material found was infinitesimal and was not pursued further.

It seemed possible that although no cholesterol could be obtained by repetition of the Kazakova technic, it might yet be present in the mucin complex. Accordingly 817 mg. of mucin were hydrolyzed with 2N KOH for 2 hours and extracted with ether. No sterol could be demonstrated in the extract.

DISCUSSION

No evidence was found of the presence of cholesterol as a constituent of human salivary mucin. There is inconclusive evidence suggesting the presence of about 1 mg. of a sterol, other than cholesterol, in the ethanol extract of 2.43 Gm. of dried mucin.

The recovery experiment is significant as it shows that the presence of added cholesterol in the saliva/acetic acid/acetone mixture causes some 23 per cent to be adsorbed on the mucin as it precipitates. This adsorbed cholesterol is readily removed by washing with cold chloroform and the mucin,

washed in that way, is free from cholesterol. During the normal experiments, this washing was omitted, which suggests that the traces of lipids found might well be present due to adsorption from saliva on the precipitated mucin.

Krasnow[8] has reported on the presence of free cholesterol in saliva.

It is concluded that the human salivary mucin, precipitated and prepared, as described, contains no demonstrable amount of cholesterol.

SUMMARY

1. Methods for the collection of saliva and the precipitation of mucin from it are described and discussed.

2. The claim that cholesterol is an integral component of human salivary mucin was examined by experimental methods, including the original technic of Kazakova.

3. It is concluded that the mucin precipitated by us, from saliva collected as described, contains no cholesterol capable of being identified by the usual chemical means.

REFERENCES

1. Kazakova, M. E.: Salivary Mucin as a Glucolipoprotein, *Biokhimiya* **17**: 195, 1952.
2. Knox, K. W.: Observations on the Salivary Mucolytic Enzymes, *J. D. Res.* **32**: 367, 1953.
3. *Idem:* Observations on the Action of Mucolytic Enzymes on Salivary Mucoid, *J. D. Res.* **32**: 374, 1953.
4. Knox, K. W., and Still, J. L.: Observations on the Salivary Mucoids, *J. D. Res.* **32**: 379, 1953.
5. Clement, A. J.: The Chemical Composition of Salivary Mucin (Mucoprotein), *J. D. A. South Africa* **6**: 277, 1951.
6. Inouye, J. M.: Biochemical Studies on Salivary Mucin, *J. D. Res.* **10**: 7, 1930.
7. Morton, R. A.: The Application of Absorption Spectra to the Study of Vitamins, Hormones and Co-Enzymes, London, 1942, Adam Hilger, Ltd., p. 33.
8. Krasnow, F., and Oblatt, E. B.: Salivary Cholesterol, *J. D. Res.* **16**: 151, 1937.

EGG YOLK REACTIONS BY ORGANISMS FROM THE ORAL CAVITY OF MAN

GENEVIEVE D. ROTH, D.D.S., AND HUGH I. MYERS, PH.D.

University of Kansas City, Kansas City, Mo.

MANY bacteria are capable of producing fat-splitting enzymes, but to date none have been recovered from the human oral environment. This report deals with the occurrence of lecithinase and a lecithinase-like reaction on egg yolk plates by cultures taken from normal and inflamed gingival sulci and a study of the representative bacterial groups which were isolated. Egg yolk plates were selected because of their use in the identification of lecithinase and allied enzymes.

LITERATURE

Alpha toxin (lecithinase) from *Clostridium welchii* is the chief cause of the toxemia of gas gangrene.[1] It was first recognized by Nagler[2] and Seiffert[3] who independently reported an opalescence in human serum developed by *Clostridium welchii*. This is known as the "Nagler" reaction. Macfarlane, Oakley, and Anderson[4] developed a crude lecithovitellin test from egg yolk which is known to contain lipoproteins, lecithovitellin, and lecithovitellinin. Using the toxin in the egg yolk solution, a layer of fat arose to the surface. When tested serologically, the toxin proved to be a single factor causing the Nagler reaction, egg yolk reaction, and hemolysis of sheep red blood cells. It occurred only in the presence of ionized calcium.

Macfarlane and Knight[5] found the optimum pH to be 7.0 to 7.6 and that fluoride, citrate, and phosphate inhibited the reaction. Determination of breakdown products revealed phosphocholine and a diglyceride and it was suggested that the lecithinase be called lecithinase "C" as it is a phosphatase. Hemolysis is caused by a structural alteration when lecithin is broken down in the red cell wall.

van Heyningen[6] developed an egg yolk method which could be used colorimetrically for α toxin measurement, studied separation of α and θ toxin and experimented on media for large scale production of the toxin of *Clostridium welchii*, type A.

Crook[7] stated that the calcium ion was necessary for the lecithinase reaction. Hayward[8] suggested the use of the Nagler reaction for a rapid identification of *Clostridium welchii*, but Weed[9] stated that the serum-opacity test was nonspecific for *Clostridium welchii*. McClung, Heidenreich and Toabe[10] developed a medium, similar to that used by Hayward but simpler to prepare,

This study was supported (in part) by funds provided under contract A.F. 18(600)901 with the U.S.A.F. School of Aviation Medicine, Randolph Field, Texas.

Received for publication Sept. 2, 1955.

for the Nagler plate reaction for identification of certain clostridia. The egg yolk plate for presumptive diagnosis of various clostridia was developed by McClung and Toabe[11] and Bard and McClung[12] separated the crude toxins from *Clostridium novyi* and *Cl. hemolyticum*.

van Heyningen and Bidwell[13] reported the antigen-antibody reaction of α toxin from *Clostridium welchii*. Zamecnik, Brewster, and Lipmann[14] developed a manometric method for measuring *Clostridium welchii* lecithinase. McGaughey and Chu[15] used the egg yolk plate to study aerobic sporing bacilli. Other clostridia were studied for lecithinase activity, *Clostridium novyi* by Oakley, Warrack and Clark,[16] *Clostridia oedematiens* and *sordelli* by Macfarlane,[17] and *Clostridium haemolyticum*[18] and *Clostridium bifermentans* by Miles and Miles,[19] while Monsour and Colmer[20] studied the genus *Serratia* and its reaction to egg yolk.

Kass, Lichstein and Waishren[21] were interested in the occurrence of hyaluronidase and lecithinase in relation to virulence in *Clostridium welchii*. Scheff and Awny[22] found an increased lecithinase activity in splenic dyscrasias. Evans and Prophet[23] examined lecithinase production by clostridia isolated from human teeth. Gillespie and Alder,[24] in a study of coagulase-positive staphylococcus, noted an appearance on egg yolk agar similar to that produced by lecithinase. Chemical examination of the curd formed on the egg yolk revealed that it was not a lecithinase "C" or a phosphatase, although it may be caused by a lipase activity against triglycerides of higher fatty acids.

Johansson[25] modified the egg yolk medium by adding sodium azide for isolation of lecithinase production by anaerobes in feces.

MATERIALS

The basic test for lecithinase and allied activity was accomplished with an egg yolk plate used either as a first sub-culture or as a primary plate. This was prepared by adding 10 per cent sterile egg yolk to Difco Blood Agar Base which had been melted and then cooled to 50° C. The reaction on the plate resembled that described by McClung.[11] Streak plates and pour plates were utilized. All plates were incubated anaerobically (hydrogen 90 per cent, carbon dioxide 10 per cent) at 37° C. and observed for 10 days. Lecithinase-like activity was indicated as areas of precipitation with or without iridescence or clearing of the medium around the colonies. Isolations were made from selected lecithinase positive areas.

Liquid primary media included Difco Brewer Thioglycollate Medium (B236) with added crystal violet prepared according to the Manual of Pure Culture Study[26] as was the alkaline egg broth. Synthetic medium was prepared as suggested by Boyd, Logan, and Tytell.[27] Robertson's Meat Broth is a chopped beef heart medium.[28] The iron-litmus milk was according to Robinson and Stovall[29] and was made by adding iron by hydrogen to Difco Litmus Milk.

Isolation procedures necessitated the use of many test materials. To B.B.L. Trypticase Agar Base the sugars were added for fermentation studies. The coagulase test was done on human plasma. Catalase was read on the egg

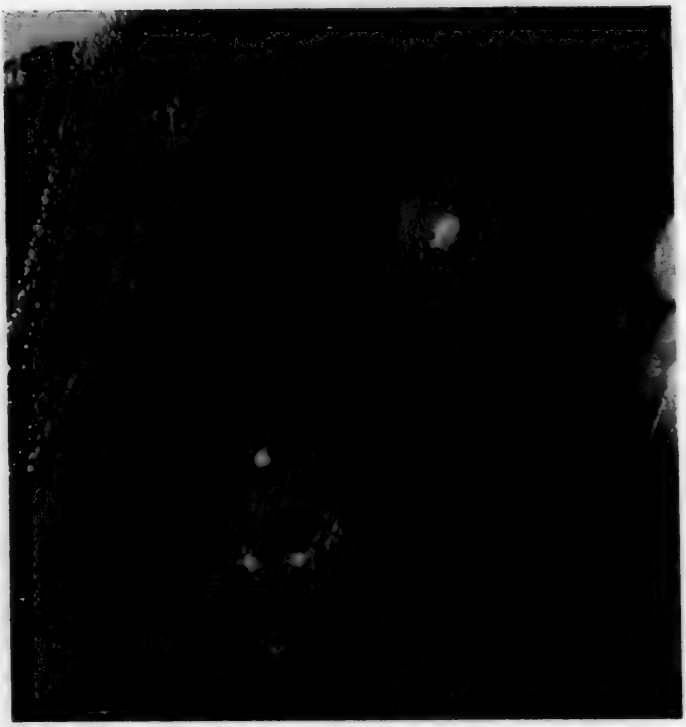


Fig. 1.—Lecithinase-like reaction in mixed culture due to *Micrococcus pyogenes* var. *albus*.

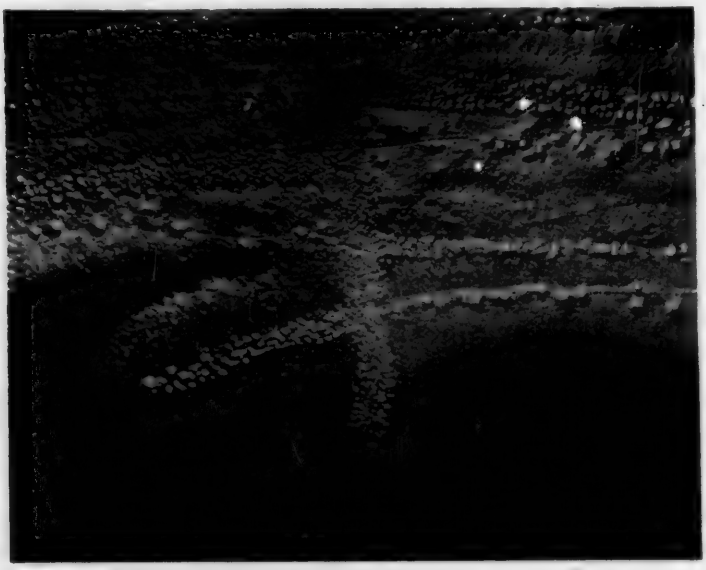


Fig. 2.—*Clostridium welchii* lecithinase reaction on egg streak plate.

plates by flooding the plate with 3 per cent H_2O_2 diluted with equal parts water. Gelatin was made by using 10 per cent nutrient gelatin in Brewer's Thioglycollate medium. In all blood plates, 10 per cent human citrated whole blood was used.

METHODS

Sixteen normal patients were selected to serve as controls. These normals had no pocket formation and no inflammation of the supporting structures. Caries was not considered.

Experimental Group I included 33 patients, all showing inflammatory changes (periodontitis, pericornitis, mild gingivitis, periodontal abscess, or periodontitis complex).

Primary cultures were taken from both groups by inserting sterile paper points in the gingival sulci and then inoculating one of the liquid media (Table I) and transferring, after 24 hours, to anaerobic egg pour plates (first subculture).

TABLE I
LECITHINASE-LIKE ACTIVITY FROM NORMAL AND INFLAMMATORY CASES

PRIMARY CULTURE	FIRST SUB-CULTURE	EXPERIMENTAL					CONTROL				
		LECITHINASE-LIKE ACTIVITY			CASES		LECITHINASE-LIKE ACTIVITY			CASES	
Group I		+	†	–	NO.	%	+	†	–	NO.	%
Thioglycollate with cry. violet 1:200,000	Egg Pour Plate	1	0	0	1		0	0	1	1	
Synthetic Medium	Egg Pour Plate	6	0	2	8		3	0	4	7	
Robertson's Meat Broth	Egg Pour Plate	2	0	0	2						
Alkaline Egg Broth	Egg Pour Plate	1	0	1	2						
Iron-Litmus Milk	Egg Pour Plate	12	3	5	20		6	1	1	8	
		22	3	8	33	67	9	1	6	16	56
Group II											
Egg Pour Plates		15	0	0	15	100					

Experimental Group II was made up of 15 patients with inflammation. The inoculum was secured on sterile paper points which were rinsed in broth which was used immediately to make direct primary egg pour plates.

RESULTS

The basic results of the use of the various primary culture media are given in Table I. It is readily apparent that positive lecithinase-like activity was secured from both normal and inflamed mouths.

In some cases the lecithinase activity appeared as only a precipitation zone, but in general it was combined with an iridescence.[11] The zone varied from one under the colony, to a narrow band, or to a wide reaction zone and was constant with the isolate. This was usually accompanied by a clearing of the medium (Fig. 1).

In mixed cultures, the reaction was slow in developing (depending on the primary culture method) and often required 6 to 10 days. Positive areas

have been seen to develop as late as 14 days. After isolation, the reaction time was greatly shortened; the clostridia developed wide zones within 24 hours (Fig. 2).

Isolations of lecithinase-positive organisms were extremely difficult to achieve, and those secured were often discarded when they failed to produce the lecithinase-like reaction in pure culture. In the pure studies the reaction was often variable, appearing around a few colonies on the plate (Fig. 3) or areas which when studied showed no morphologic deviations from the negative areas on the same plate.

Fig. 3.—Egg yolk reaction occurring only in isolated areas on pure plate.

Some observations of the isolates revealed the organisms and their relationships as indicated below. Three anaerobic streptococci were found which were lecithinase positive. Six *Micrococcus (pyogenes* var. *albus* and var. *aureus)* gave an egg yolk reaction; many others were negative. This has been reported by Gillespie and Alder.[24] Clostridial isolates included 2 *C. bifermentans* and 2 *C. perfringens* which gave their typical reaction.[11] With regard to lecithinase, the actinomyces studied were positive, as was 1 culture of *Bacteroides melaninogenicus,* while strains of *E. coli,* fusiforms and a group of 9 lactobacilli from the American Type Culture Laboratory were negative.

Probably the most interesting phase of the work was reached when it was discovered that a pleomorphic rod (Corynebacterium) from a positive area, negative on pure sub-culture, was able to regain its lecithinase-like ability when recombined with a gram-positive anaerobic streptococcus (also negative on pure sub-culture) secured from the same patient (Fig. 4).

Subsequently 2 distinct groups of Corynebacterium were isolated. The first was catalase-positive, highly proteolytic, and only slightly saccharolytic and lecithinase negative. However, these were able to produce the lecithinase-like reaction when cross streaked on the egg yolk plates either with the anaerobic lecithinase negative streptococcus or a lecithinase-negative lactobacillus. This symbiotic activity suggests interesting possibilities, but greatly complicates isolation procedures and attempts to correlate findings.

Fig. 4.—Symbiotic production of lecithinase-like reaction. Vertical streak of anaerobic streptococcus is made first. Horizontal streak of corynebacteria is made from right to left. It shows the pure culture reactions to the point of intersection and symbiotic reaction as the cultures are blended to complete the stroke.

The second group of Corynebacterium was catalase negative, nonproteolytic and tended to utilize more sugars. These were either lecithinase positive or variable in pure culture. Altering pH or changing nutrients brought about only slight changes in the reactions of the members of the two groups.

DISCUSSION AND SUMMARY

The high percentage of positive findings on the egg plates indicate the presence of a fat-splitting enzyme or enzymes produced by the microorganisms of the mouth. It is a well-established fact that clostridia produce a lecithinase C, and this present experiment suggests that while these organisms may be found, they are not frequent inhabitants. However, it is apparent that the plate reaction, considered by some to be a valid test for lecithinase C, is produced by other organisms and by symbiotic combinations of organisms. It is also of interest that, although many *Micrococcus pyogenes* var. *albus* and var. *aureus*

were found, only a few of them were able to produce the lecithinase-like reaction. In view of the fact that there is no valid test for pathogenicity of the staphylococci, exploration along this line might prove rewarding.

In order to correlate the presence of this enzyme or enzymes with a specific clinical entity, it will be necessary to isolate from each patient all organisms yielding the reaction. The symbiotic relationship will complicate the procedures as will the individual cultural requirements of the various organisms. Also necessary will be a study of the exact chemical nature of each enzyme system and its in vivo action. Normals will have to be caries free, as it is possible that these enzymes might be related to the carious process which is known to have a fatty degenerative phase. However, it is apparent that lecithinase-like producing organisms are found in the oral cavity. Their contribution to the inflammatory entities is unknown at the present time.

REFERENCES

1. van Heyningen, W. E.: Bacterial Toxins, Springfield, Ill., 1950, Charles C Thomas, Publisher.
2. Nagler, F. P. O.: Observations of a Reaction Between the Lethal Toxins of *Clostridium welchii*, Type A, and Human Serum, *Brit. J. Exper. Path.* **20**: 473-485, 1939.
3. Seiffert, G.: Eine Reaktion Menschlicher Sera Mit Perfringenstoxin Zeitschr, *Immunitatsf.* **96**: 515-520, 1939.
4. Macfarlane, R. G., Oakley, C. L., and Anderson, C. G.: Haemolysis and the Production of Opalescence in Serum and Lecithovitellin by the α Toxin of *Clostridium welchii*, *J. Path. Bact.* **52**: 99-103, 1941.
5. Macfarlane, M. A., and Knight, B. C. J. A.: Biochemistry of Bacterial Toxins. 1. The Lecithinase Activity of a *Clostridium welchii* Toxin, *Biochem. J.* **35**: 884-902, 1941.
6. Van Heyningen, W. E.: The Biochemistry of the Gas Gangrene Toxins, 1. Estimation of the α Toxin of *Clostridium welchii*, Type A, *Biochem. J.* **35**: 1246-1256, 1941a. 2. Partial Purification of the Toxins of *Clostridium welchii*, Type A, Separation of α and θ Toxins, *Biochem. J.* **35**: 1257-1269, 1941b. 3. Development of a Medium Suitable for the Large Scale Production of the Toxins of *Clostridium welchii*, Type A, *Biochem. J.* **42**: 127-130, 1948
7. Crook, E. M.: Nagler Reaction: The Breakdown of Lipo-Protein Complexes by Bacterial Toxins, *Brit. J. Exper. Path.* **23**: 37-55, 1942.
8. Hayward, N. J.: The Rapid Identification of *Clostridium welchii* by Nagler Tests in Plate Culture, *J. Path. Bact.* **55**: 285, 1943.
9. Weed, L. A., and Minton, S.: The Nonspecificity of the Serum Opacity Test for *Clostridium welchii*, *J. Lab. & Clin. Med.* **28**: 1251, 1943.
10. McClung, L. S., Heidenreich, P., and Toabe, R.: A Medium for the Nagler Plate Reaction for the Identification of Certain Clostridia, *J. Bact.* **51**: 751-752, 1946.
11. McClung, L. S., and Toabe, R.: The Egg Yolk Plate Reaction for the Presumptive Diagnosis of *C. sporogenes* and Certain Species of the Gangrene and Botulinum Groups, *J. Bact.* **53**(2): 139-147, 1947.
12. Bard, R. C., and McClung, L. S.: Biochemical Properties of the Toxins and *Clostridium novyi* and *C. hemolyticum*, *J. Bact.* **56**(5): 665-670, 1948.
13. Van Heyningen, W. E., and Bidwell, E.: The Biochemistry of the Gas Gangrene Toxins. 4. The Reaction Between the α Toxin (Lecithinase) of *Clostridium welchii* and Its Antitoxin, *Biochem. J.* **42**: 130-140, 1948.
14. Zamecnik, P. C., Brewster, L. E., and Lipmann, F.: A Manometric Method for Measuring the Activity of the *Clostridium welchii* Lecithinase and a Description of Certain Properties of This Enzyme, *J. Exper. Med.* **85**: 381-394, 1947.
15. McGaughey, C. A., and Chu, H. P.: The Egg-Yolk Reaction of Aerobic Sporing Bacilli, *J. Gen. Microbiol.* **2**: 334-341, 1948.
16. Oakley, C. L., Warrack, G. H., and Clark, P. H.: The Toxins of *Clostridium oedematiens* (Cl. novyi), *J. Gen. Microbiol.* **1**: 91-107, 1947.
17. Macfarlane, M. G.: The Biochemistry of Bacterial Toxins. 3. Identification and Immunological Reactions of Lecithinase Present in *Clostridium oedematiens* and *Clostridium sordelli* Toxins, *Biochem. J.* **42**: 590-595, 1948b.
18. Macfarlane, M. G.: Biochemistry of Bacterial Toxins. 1. Lecithinase Activity of *Clostridium haemolyticum* Toxin, *Biochem. J.* **47**(3): 267-270, 1950.

19. Miles, E. M., and Miles, A. A.: The Lecithinase of *Clostridium bifermentans* and Its Relation to the *a* Toxin of *Clostridium welchii*, *J. Gen. Microbiol.* **4**: 22-35, 1950.
20. Monsour, V., and Colmer, A. R.: The Action of Some Members of the Genus Serratia on Egg Yolk Complex, *J. Bact.* **63**(5): 597-603, 1952.
21. Kass, E. H., Lichstein, H. C., and Waishren, B. A.: Occurrence of Hyaluronidase and Lecithinase in Relation to Virulence in *Clostridium welchii*, *Proc. Soc. Exper. Biol. & Med.* **58**: 172-175, 1945.
22. Scheff, Geo. J., and Awny, A. J.: Lecithinase Activity in Splenic Dyscrasias, *Am. J. Clin. Path.* **19**: 615-620, 1949.
23. Evans, D. G., and Prophet, A. S.: Examination of Strains of *Clostridium welchii* Isolated From Teeth, *Brit. Dent. J.* **91**: 199, 1951.
24. Gillespie, W. A., and Adler, V. G.: Production of Opacity in Egg-Yolk Media by Coagulase-Positive Staphylococcus, *J. Path. & Bact.* **64**: 187-200, 1952.
25. Johansson, K. R.: A Modified Egg Yolk Medium for Detecting Lecithinase Producing Anaerobes in Feces, *J. Bact.* **65**: 225, 1953.
26. Manual of Method for Pure Culture Study of Bacteria, Soc. of Am. Bacteriologists, Geneva, N. Y., 1946, Biotech. Publications.
27. Boyd, M. J., Logan, M. A., and Tytell, A.: Growth Requirements of *Clostridium welchii*, *J. Biol. Chem.* **174**: 1013-1025, 1948a.
28. Hartles, R. L.: Personal Communication, University of Liverpool, School of Dental Surgery, Liverpool, England.
29. Robinson, C. L., and Stovall, W. G.: Recommended Procedure for the Laboratory Identification of *Clostridium welchii*, *Am. J. Clin. Path.* **7**: 172-183, 1937.

A COMPARATIVE STUDY OF HUMAN SERUM AND SALIVARY ANTIBODY TITERS IN CASES OF BRUCELLA MELITENSIS INFECTIONS

M. G. WHEATCROFT*

U. S. Naval Medical Research Unit No. 3, Cairo, Egypt

POLLACI and Ceraulo,[1] in 1909, demonstrated agglutinating antibodies in the salivas of 20 patients with brucella infections. The reaction was shown to be specific when tested with *Shigella dysenteriae*, 2 streptococci, *Micrococcus albus* and *aureus* and 1 tetragenes species.

The following study was planned to determine the constancy of the brucella antibody in the saliva in a large group of patients in an area where brucellosis is endemic and to compare salivary titers with serum titers by the complement-fixation and the tube agglutination test.

METHODS AND MATERIALS

Seventy-three patients with brucella infection and a control group of 15 individuals were used in this investigation. The patients were receiving treatment for brucellosis and were being studied at the Naval Medical Research Unit No. 3, Cairo, Egypt. The control group was composed of American and Egyptian laboratory workers with negative serology and no clinical signs of brucella infection.

The saliva and serum samples were collected before breakfast the same day. Salivary flow was stimulated by chewing paraffin and was collected in clean bottles. The blood samples were placed in sterile tubes and allowed to clot at room temperature, ringed, centrifuged, and the serum withdrawn. All samples were examined the same day when possible, otherwise, they were frozen and stored in the frozen state until used. All serum and saliva samples were examined by the complement-fixation or the tube agglutination method. The saliva samples used in the complement-fixation technic were filtered through coarse paper, using negative pressure. Saliva samples used in the agglutination test were filtered through gauze to remove large particles and centrifuged at 2,000 r.p.m. for 15 minutes. The supernate was withdrawn for the test.

The complement-fixation test employed was as follows:

Reagents.—Two and one-half per cent suspension sheep cells in Alsever's solution, 3 units of hemolysin, 2 units of guinea pig complement, sera or saliva diluted with veronal buffer as tabulated in Table I. Total volume in each tube was 1.0 ml.

The opinions and assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

Received for publication Sept. 12, 1955.

*Present address: U. S. Naval Dental School, Bethesda, Md.

TABLE I

DISTRIBUTION OF COMPARATIVE SERUM AND SALIVARY TITERS BY THE COMPLEMENT-FIXATION TEST

SALIVA DILUTIONS	SERUM DILUTIONS											ΣY
	NEG.	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:10240	
Neg.	9	1	2	1	2	7	5	1				28
1:2	1	–	2	1	3	7	10	2	5			31
1:4						2	3	6	2	3	3	19
1:8									1	1	1	3
1:16										1	1	2
1:32										2	1	3
1:64										1	–	1
ΣX	10	1	4	2	5	16	18	9	8	8	6	87

Correlation coefficient = .674.
Probable error = ± .039.

Procedure.—Inactive at 56° C. for 20 minutes, fix at 37° C. in water bath for 60 minutes, add sheep cells with hemolysin (sensitized), incubate at 37° C. in water bath for 30 minutes, and then read.

The last tube showing 50 per cent hemolysis, in both the saliva and serum tubes, was used to determine the titer for each test. All saliva or serum samples showing anti-complementary readings were not included in the results.

The standard tube agglutination method was used. The total volume in each tube was 1.0 ml. Saliva and serum serial dilutions were made with sterile saline; the dilutions used are shown in Table II. The serum dilution in the first tube was 1:80; this titer was considered as diagnostic. Antigen in 0.5 ml. amounts was added to each tube and the test tubes incubated in a water bath at 56° C. for 2 hours. The tubes were placed in the refrigerator at 5° C. overnight and read the following morning. The last tube showing sedimentation (agglutination) was used as an indicator of the titer in both the serum and saliva samples.

TABLE II

DISTRIBUTION OF COMPARATIVE SERUM AND SALIVARY TITERS BY THE TUBE AGGLUTINATION METHOD

SALIVA DILUTIONS	SERUM DILUTIONS									ΣY
	NEG.	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240	
Neg.	15	--	--	1	3	7	5	6	2	39
1:2		2	1	1	6	9	7	10	11	47
1:4				1	1	2	3	6	13	26
1:8						2	--	--	11	13
1:16							--	--	3	3
1:32								1	2	3
1:64									1	1
ΣX	15	2	1	3	10	20	15	23	43	132

Correlation coefficient = .524.
Probable error = ± .052.

In the complement-fixation tests the dilution of antigen was determined by anti-complementary tests. The antigen dilutions were prepared as follows: 1:4, 1:6, 1:8, 1:10, etc. The second negative tube was considered the proper dilution for use in the test. This gave a maximum concentration of antigen, which was necessary due to the low antibody content of the saliva.

The antigen used in these determinations was *Brucella melitensis*. The original isolation was obtained from a human blood culture. Heat-killed, phenolized preparations were used and the working antigen was prepared by diluting the bacterial suspension with sterile phenolized saline to match a No. 3 MacFarlain nephelometer. The usual controls were used in all agglutination and complement-fixation tests. Serum and salivary controls included 3 tubes to correspond to the first 3 serial dilutions.

Attempts were made on 35 saliva samples to increase the available antibody by decreasing the saliva volume. Visking bags were filled to 15-20 ml. volumes. The bags were tied securely and suspended horizontally in front of electric fans or forced air ducts in order to evaporate the material passing through the membrane. Two to 3 hours were required to reduce the saliva volume to approximately 2 ml. The concentrated sample was serially diluted and tested by the tube agglutination method.

RESULTS

Table I shows the distribution of serum and saliva titers by the complement-fixation test. The total number of paired observations was 87. The total number of observations at each dilution of saliva is indicated by ΣX. Each XY co-ordinate represents the number of paired serum and saliva titers observed. For example, 10 in the 1:512 1:2 co-ordinate, signifies there were 10 pairs of observations when the saliva had a titer of 1:2 and the serum had a titer of 1:512.

Correlation analyses were calculated from the data in Table I, to determine whether there was an association between the rise in titer of saliva and serum. The method used has been described by Pearl.[2] The correlation coefficient (r) = .674 was obtained, with a probable error of ± .039.

Table II shows the distribution of comparative paired observations of serum and saliva by the tube agglutination tests. The correlation coefficient obtained from the data in Table II was .524 with a probable error of ± .052.

Although the correlation shown between the serum and saliva by the complement-fixation tests was not as high as that obtained by the tube agglutination method, approximately 50 per cent of rising titers showed a statistical association.

The complement-fixation data in Table I, shows there were 28 negative salivas and only 10 negative sera. Of the total salivas, 18 per cent were negative when the corresponding sera were positive for brucella antibody. In one case there was a positive saliva and a negative serum.

The agglutination data of Table II shows similar results. The tests showed 21 per cent negative salivas while the corresponding sera were positive; however, there was no paired negative serum and positive saliva. If the data from the complement-fixation and agglutination tests are combined, approximately 80 per cent of the salivas were positive for brucella antibody when the sera were diagnostically positive (1:80 or over).

When the saliva was concentrated, an increase in antibody titer could be demonstrated by the tube agglutination method. Concentration of the saliva

was not feasible when tested by the complement-fixation test due to a corresponding concentration of anti-complementary factors. Negative salivas, from patients with brucellosis with diagnostic serum titers, were shown to contain antibody when concentrated. The antibody present was not in sufficient amount to show agglutination unless concentrated. Salivary antibodies, in control patients, could not be demonstrated in the routine or concentrated salivas.

DISCUSSION

The reading and evaluation of the salivary agglutination tubes varied somewhat from the serum tubes. Routinely, the supernatant fluid should be clear in a completely agglutinated tube. Since many of the salivary samples are turbid, more dependable readings can be obtained if the amount and type of sediment in the tube is used as the criterion of agglutination. Fig. 1 shows the varying amounts of sedimentation (agglutination) occurring in saliva agglutination.

The higher correlation coefficient obtained in the complement-fixation data was probably due to the following: The serial dilutions in the agglutination tests were not high enough to reflect the end points of several sera. Table II shows 43 sera having a 1:10,240 dilution (the greatest number at any 1 dilution) thus a normal distribution was not represented.

This study indicated that the tube agglutination test is the method of choice for the determination of salivary antibody in *Brucella melitensis* infections. The complement-fixation test is impractical because of anti-complementary substances in the saliva. Debris was a problem in many of the salivas; filtration through Seitz, Coors porcelain or sintered glass filters completely removed the antibody when present in small amounts. There was a slight decrease in the titer even when the saliva was filtered through paper as compared to centrifugation.

Although the coefficient of correlation showed an associated rise in titer in the sera and saliva there is considerable variation among individuals. This would be expected, due to the individual physiologic differences in the capabilities of salivary gland transfer of antibodies from serum to saliva. It was proposed by Coleman and Appleman,[3] in a study of the antibodies of syphilis, that the individual differences may be reflected by the length of time the infection had been present and whether treatment or re-infection had occurred. Salivary antibodies may appear at a later time than in the serum and may disappear earlier. If this is true it may explain the weak or negative salivary titer when the serum titer is high. It was not possible in this investigation to study this time relationship due to difficulties of obtaining follow-up sera and saliva samples. Although the concentration technic was not tried on all the salivas it is felt that the results of 35 cases indicate that antibody can routinely be demonstrated in the saliva when the serum has a titer of 1:80 or over.

This study shows a correlation in serum and salivary brucella titers. From this correlation it is more probable that antibodies are transferred from the serum to the saliva rather than the assumption that the salivary gland is a

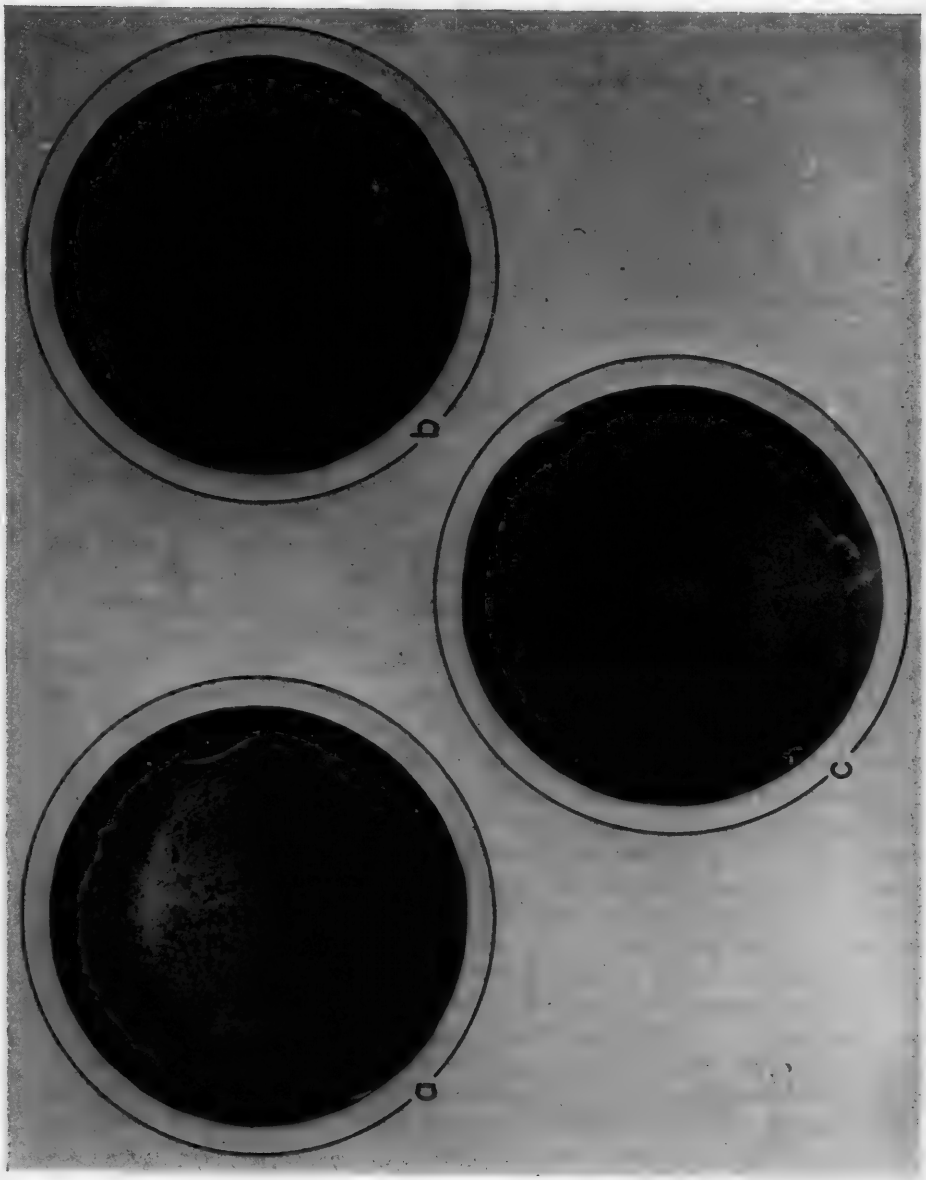


Fig. 1.—Sedimentation in tube agglutination tests of saliva. *a*, Complete or +++ agglutination. *b*, Partial or ++ agglutination. *c*, No agglutination. The small button of "soft" antigen can be seen in the bottom of the tube.

site of antibody formation. Since it appears to be an antibody transfer, then the saliva must be considered as a vehicle for antibody loss as the flow is continuous and reabsorption in a significant amount is improbable.

Because of the 20 per cent false negative salivas (negative saliva with positive serum), it is felt that the routine use of saliva for diagnosis of brucellosis is not practical at this time. However, if further studies on filtration and concentration technics are successful, the saliva may prove a useful adjunct in the diagnosis of brucellosis.

SUMMARY

Two hundred and nineteen saliva and sera samples, collected from 73 patients with *Brucella melitensis* infections, were examined by the complement-fixation and tube agglutination technic to form a comparative study of antibody titers.

Eighty per cent of the salivas were positive for brucella antibody when the sera contained a titer of 1:80 or over. Salivas from control individuals were consistently negative.

Concentration of the negative salivas from clinical patients with a serum titer of 1:80 or over showed the antibody to be consistently present, when tested by the tube agglutination method. Concentration of the saliva of control individuals gave negative results. It was not possible to use the complement-fixation test on concentrated salivas because of the concentration of anti-complementary factors in the saliva.

A statistical association in the amount of antibody in the sera and saliva from each patient was shown by correlation analyses. In the complement-fixation tests the correlation coefficient obtained was .674 and in the agglutination tests the correlation coefficient was .524.

Tables are presented showing the distribution of the comparative titers by the complement-fixation and agglutination tests.

I wish to thank Lt. (jg) W. R. Furrey, MSC, USN, and DTC C. O. Boland, USN, for technical assistance. I also appreciate the assistance given by personnel of the Virology and Bacteriology Departments for preparation for some of the reagents used in this study.

REFERENCES

1. Pollaci, G., and Ceraulo, S.: The Agglutinating Properties of Several Body Fluids During Malta Fever, *Centralbl. f. Bakteriol.* (1) 52 (2): 268, 1909.
2. Pearl, R.: Introduction to Medical Biometry and Statistics, Philadelphia, 1941, W. B. Saunders Company, pp. 419-425.
3. Coleman, R. D., and Appleman, M. D.: Antibodies of Syphilis in Saliva and Serum, *J. D. Res.* **32**: 294, 1953.

EFFECTS OF BUFFERED SOLUTIONS OF SODIUM FLUORIDE AND STANNOUS FLUORIDE ON THE SOLUBILITY OF POWDERED ENAMEL USING REPEATED DECALCIFICATION

ROBERT H. WALSH, PH.D., WILLIAM H. NEBERGALL, PH.D., JOSEPH C. MUHLER, D.D.S., PH.D., AND HARRY G. DAY, SC.D.

The Department of Chemistry, Indiana University, Bloomington, Ind.

CONFLICTING reports have recently appeared in the literature regarding the effectiveness of different fluorides in reducing the acid solubility of powdered dental enamel and whole teeth at low pH levels. Bibby,[1] Phillips and Muhler,[2] and Palmer, Overstreet, and Sacks,[3] using gravimetric procedures, observed that the protection of enamel against acid solubility by sodium fluoride was enhanced by lowering the pH of the protecting solutions. Clinical studies[4-6] failed to corroborate the results of these in vitro investigations. Using a volumetric procedure,[7] Muhler and Day[8] concluded that the effectiveness of sodium fluoride and stannous fluoride in decreasing powdered enamel solubility is substantially independent of the pH at which the fluoride is applied to the enamel when only the decalcification values are compared. It was observed in these studies, however, that at the lower pH values appreciable decalcification of the enamel resulted from treatment with the sodium fluoride solutions.[8] This did not occur with the stannous fluoride solutions at the same pH. This same observation has been found in experimental dental caries studies in the rat,[9] in which it was found that at a low pH sodium fluoride and stannous fluoride appear equal in their anticariogenic effectiveness, but at the lower pH values extreme erosion of the molar teeth occurred in the sodium fluoride group, thus making the diagnosis of dental caries in this group quite difficult. However, experimental hamster dental caries studies[10] indicated that pH was of little importance in increasing the effectiveness of fluoride solutions.

It was reported[11] from this laboratory that stannous fluoride given to rats as a supplement in the water at pH 2.6 was almost twice as effective in reducing the incidence of carious lesions as the same amount of stannous fluoride when present in the drinking water at pH 3.6. In addition, a 55 per cent reduction in the incidence of dental decay was obtained in a recent clinical study[12] in which stannous fluoride at pH 2.6 was topically applied to the teeth of school children following the usual clinical practices.

The fact that stannous fluoride is quite effective as an anti-cariogenic agent when administered at low pH levels obviates the need for further in vitro studies regarding the relationship of pH to fluoride protection. Using repeated decalcifications, an attempt has been made to evaluate more critically the protection afforded powdered enamel by sodium fluoride and stannous fluoride at lower pH levels and in neutral solutions.

Received for publication Aug. 25, 1955.

EXPERIMENTAL

The method of Muhler, Boyd, and Van Huysen[7] for the evaluation of fluorides in decreasing the solubility of dental enamel includes the measurement of the decalcification that takes place during the treatment of the enamel by the protecting solution. When acidulated solutions of sodium fluoride are used to protect powdered enamel, considerable decalcification occurs during the protective treatment. This could mean that if sodium fluoride were evaluated clinically, using a low pH, some surface dissolution might occur. When neutral solutions of sodium fluoride are employed, negligible amounts of phosphorus are dissolved, indicating little dissolution of the enamel. The question arises as to whether or not this initial loss of phosphorus during protection of enamel when the solution has a low pH decreases the availability of this element for the subsequent decalcification step in the evaluation procedure. A solution to this problem was sought by comparing the loss of phosphorus from powdered enamel samples protected by sodium fluoride solutions buffered at pH 4.0 with sodium acetate-acetic acid to the loss of phosphorus from enamel treated with the buffer solution containing no fluoride. It has been found that by making 3 successive decalcifications with fresh decalcifying solutions, a more critical evaluation of the protection of an enamel sample may be made than when only 1 decalcification is employed.

The procedure involved the treatment of a 100 mg. sample of 60 mesh dental enamel[7] with 5 ml. of a fluoride solution buffered at pH 4.0 with a sodium acetate-acetic acid solution. After being shaken for 20 minutes, the mixture was centrifuged and the supernatant was decanted and analyzed for phosphorus. The loss of phosphorus during the protective treatment was taken as a measure of the decalcification by the protecting solution. Next, a 10 ml. portion of decalcifying solution, consisting of 0.2 N acetic acid adjusted to pH of 4.0 by adding sodium hydroxide, was added to the protected enamel sample and the mixture was shaken for 20 minutes. Following centrifugation, the supernatant was decanted and analyzed for phosphorus. A second 10 ml. portion of acid was added to the same sample of enamel and decalcification was carried out as before. This procedure was repeated a third time. The amounts of phosphorus dissolved during the protective treatment and the 3 successive decalcifications are given in Table I. The control was run in the same manner except that the fluoride was omitted.

By considering the phosphorus dissolved during the protective treatment and the first decalcification in the case of sodium fluoride applied at a pH of 4.0, one might infer that the loss of phosphorus during the decalcification is low because a considerable quantity was removed during protection, thus decreasing the total amount available. The total phosphorus liberated during the 2 treatments is 0.84 mg., as compared with 1.08 mg. when enamel was treated with water rather than sodium fluoride. A comparison of these 2 figures (0.84 and 1.08) gives a picture of very little protection by sodium fluoride at pH 4.0.

TABLE I
PHOSPHORUS DISSOLVED DURING THE PROTECTIVE TREATMENT AND THREE SUCCESSIVE DECALCIFICATIONS OF POWDERED ENAMEL

SAMPLE NUMBER	PROTECTING SOLUTION	pH	BUFFER SYSTEM	PHOSPHORUS DISSOLVED (MG./SAMPLE) DURING PROTECTION (1)	DURING FIRST DECALCIFICATION (2)	SUM OF 1 AND 2	DURING SECOND DECALCIFICATION (3)	DURING THIRD DECALCIFICATION (4)	SUM OF 2, 3, AND 4
1	1% NaF	4.0	NaOAc + HOAc	0.72	0.12	0.84	0.20	0.42	0.74
2	1% NaF	4.0	NaOAc + HOAc	0.72	0.13	0.85	0.23	0.43	0.79
3	1% NaF	4.0	NaOAc + HOAc	0.71	0.12	0.83	0.21	0.41	0.74
	Average of 1, 2, 3			0.72	0.12	0.84	0.21	0.42	0.75
4	Buffer	4.0	NaOAc + HOAc	1.01	1.35	2.36	1.23	1.17	3.75
5	Buffer	4.0	NaOAc + HOAc	0.94	1.24	2.18	1.17	1.10	3.51
6	Buffer	4.0	NaOAc + HOAc	0.96	1.37	2.33	1.31	1.19	3.87
	Average of 4, 5, 6			0.97	1.32	2.29	1.24	1.15	3.71
7	Water	7.0	None	0.00	1.16	1.16	1.09	1.05	3.30
8	Water	7.0	None	0.00	1.03	1.03	1.00	0.90	2.93
9	Water	7.0	None	0.00	1.05	1.05	1.05	0.98	3.08
	Average of 7, 8, 9			0.00	1.08	1.08	1.05	0.98	3.10

However, if one notes the fact that a second and third decalcification of enamel treated with water alone gives a loss of phosphorus equal to that of the first decalcification, it becomes obvious that the availability of phosphorus is not a factor to be considered in determining the degree of protection. Therefore, a comparison of the phosphorus values obtained for the first decalcification, 0.12 for sodium fluoride at a pH of 4.0, 1.08 for water treatment, and 1.32 for the buffered treated enamel, gives a more accurate picture of the protection afforded powdered enamel by sodium fluoride at the low pH level.

It is interesting to note, and no doubt significant, that the protection of the enamel by sodium fluoride persisted through 3 treatments with the decalcifying solution. This procedure for the evaluation of protecting substances and conditions of treatment, such as pH, promises to be more critical than procedures in which only 1 decalcification is employed.

To demonstrate the agreement in results, the data for triplicate runs are given in total in Table I rather than as averages.

The present investigation of the relationship of pH to the effectiveness of fluorides in decreasing enamel solubility differs from that reported by Muhler and Day[8] in regard to the method of pH control. In the previous report the hydrogen ion levels of the protecting solutions were adjusted (not buffered) by the addition of strong acid or base. This means that the pH of the protecting solutions might not remain constant during the treatment of the enamel. The results given in this paper, unless otherwise specified, are based upon pH values that were held constant throughout the time of the protective treatment of the enamel by employing appropriate buffer systems.

From a study of the relative effectiveness of 2 per cent sodium fluoride in reducing enamel solubility at pH 7.0 and 5.5, it was concluded by Muhler and Day[8] that the protection is substantially independent of pH. One might draw the same conclusion by examining the data given in Table II for 1 per cent sodium fluoride, since the loss of phosphorus at pH 7.0 is only slightly greater than at pH 5.1. As is shown in Table II, however, decreasing the pH of the 1 per cent sodium fluoride to a value of 4.0 causes a considerable decrease in the loss of phosphorus. The same trend is also noted with 2 per cent sodium fluoride. It is apparent, then, that in the lower pH range, the effectiveness of sodium fluoride is a function of the pH. It should be noted that 2 per cent sodium fluoride at the low pH of 2.8 causes a marked increase in the dissolution of the enamel during the protective treatment.

TABLE II
THE EFFECT OF pH UPON THE REDUCTION OF ENAMEL SOLUBILITY BY SODIUM FLUORIDE

PROTECTING SOLUTION	FLOURIDE CONCENTRATION IN PPM	pH	BUFFER SYSTEM	PHOSPHORUS DISSOLVED (MG./SAMPLE)				
				DURING PROTECTION	FIRST DECALCIFICATION (1)	SECOND DECALCIFICATION (2)	THIRD DECALCIFICATION (3)	SUM OF 1, 2, AND 3
1% NaF	4,525	7.0	None	0.05	0.60	0.72	0.79	2.11
1% NaF	4,525	5.1	NaOAc + HOAc	0.13	0.49	0.67	0.72	1.88
1% NaF	4,525	4.0	NaOAc + HOAc	0.71	0.12	0.21	0.41	0.74
2% NaF	9,050	7.0	None	0.10	0.79	0.75	0.75	2.29
2% NaF	9,050	4.0	NaOAc + HOAc	0.56	0.18	0.13	0.32	0.63
2% NaF	9,050	2.8	$KHC_8H_4O_4$ + HCl	1.19	0.24	0.08	0.19	0.51
Water	None	7.0	None	0.00	1.08	1.05	0.98	3.11
Buffer	None	4.0	NaAc + HAc	0.97	1.32	1.27	1.15	3.74

All runs were made in triplicate and the averages are reported.

Table III summarizes a study of the effectiveness of stannous fluoride in decreasing enamel solubility when used to protect enamel at different hydrogen ion concentrations. When 1 per cent and 2 per cent solutions of stannous fluoride were used in treating enamel samples, the pH of the solutions did not change from their initial values of 2.7 and 2.8, respectively. Therefore, no buffer was needed in these runs. Within experimental error, no difference was observed in the effectiveness of the unbuffered 1 per cent and 2 per cent stannous fluoride solution in decreasing enamel solubility. The 1 per cent and 2 per cent solutions of stannous fluoride were so acidic that it was necessary to add sodium hydroxide when it was desired to raise the pH of these solutions to 7.0. The pH of these fluoride solutions did not change when shaken with enamel.

A study of the data in Table III leads to the conclusion that the lower the pH, the greater the capacity of stannous fluoride for reducing powdered enamel solubility. This phenomenon may be due in part to the fact that the solubility of stannous hydroxide decreases rapidly with increase in pH. In neutral solutions the tin is almost quantitatively removed from solution as

the insoluble hydroxide. However, stannous fluoride at a concentration of 2 per cent (4,840 ppm fluoride) is still somewhat more effective at pH 7.0 than sodium fluoride at the 2 per cent (9,050 ppm fluoride) level.

An important conclusion that can be deduced from a study of the data presented in Table III concerns the resolving power of the evaluation procedure when it includes a second and third decalcification of a given sample of enamel. Previous workers measured only the phosphorus liberated during the protective treatment and first decalcification. When only these data are compared for the different stannous fluoride solutions, it appears that 1 per cent and 2 per cent solutions of stannous fluoride provides the same protection at pH 4.0 and pH 2.7 to 2.8. However, a consideration of the loss of phosphorus during the second and third decalcifications shows that these various solutions of stannous fluoride are not equivalent as regards their protective capacities.

At all hydrogen ion concentrations investigated, a 2 per cent solution of stannous fluoride is superior to a 2 per cent solution of sodium fluoride, even though the fluoride concentration of the latter is almost twice that for 2 per cent stannous fluoride.

TABLE III
THE EFFECT OF pH UPON THE REDUCTION OF ENAMEL SOLUBILITY BY STANNOUS FLUORIDE

PROTECTING SOLUTION	FLUORIDE CONCENTRATION IN PPM	pH	BUFFER SYSTEM	PHOSPHORUS DISSOLVED (MG./SAMPLE)				
				PROTECTIVE TREATMENT	FIRST DECALCIFICATION (1)	SECOND DECALCIFICATION (2)	THIRD DECALCIFICATION (3)	SUM OF 1, 2, AND 3
2% SnF_2	4,840	7.0	pH adjusted by adding NaOH	0.06	0.19	0.67	0.89	1.75
1% SnF_2	2,420	7.0	pH adjusted by adding NaOH	0.04	0.51	0.91	1.04	2.46
2% SnF_2	4,840	4.0	NaOAc + HOAc	0.08	0.00	0.18	0.52	0.70
1% SnF_2	2,420	4.0	NaOAc + HOAc	0.10	0.00	0.43	0.73	1.16
2% SnF_2	4,840	2.7	None	0.03	0.00	0.00	0.21	0.21
1% SnF_2	2,420	2.8	None	0.01	0.00	0.03	0.20	0.23
Water	None	7.0	None	0.00	1.08	1.05	0.98	3.10
Buffer	None	4.0	NaOAc + HOAc	0.97	1.32	1.27	1.15	3.71

All runs were made in triplicate and the averages are reported.

SUMMARY

The method of Muhler, Boyd, and Van Huysen[7] for the evaluation of the effectiveness of substances in reducing enamel solubility has been modified to include 3 successive decalcifications of enamel subsequent to treatment with a protecting solution. This modified procedure makes the evaluations more critical than the original method which involves only 1 decalcification.

The data presented lead to the conclusion that the protection afforded enamel by sodium fluoride and stannous fluoride is a function of the pH at which the fluoride is applied, with the greatest effectiveness appearing at low pH levels and with stannous fluoride solutions.

REFERENCES

1. Bibby, B. G.: Use of Fluorine in Prevention of Dental Caries III. A Consideration of the Effectivenes of Various Fluoride Mixtures, *J. A. D. A.* **34**: 26, 1947.
2. Phillips, R. W., and Muhler, J. C.: Solubility of Enamel as Affected by Fluorides of Varying pH, *J. D. Res.* **26**: 109, 1947.
3. Palmer, H. B., Overstreet, S., and Sacks, M.: An Evaluation of Various Techniques for Reducing Decalcification of Enamel. I. In Extracted, Erupted, Noncarious Third Molar Teeth, *J. D. Res.* **30**: 75, 1951.
4. Rickles, N. H., and Becks, H.: The Effects of an Acid and a Neutral Solution of Sodium Fluoride on the Incidence of Dental Caries in Young Adults, *J. D. Res.* **30**: 757, 1951.
5. Arnold, F. A., Jr., Dean, H. T., and Singleton, D. E., Jr.: Effect on Caries Incidence of a Single Topical Application of a Fluoride Solution to the Teeth of Young Adult Males of a Military Population, *J. D. Res.* **23**: 155, 1944.
6. Stones, H. H., Lawton, F. E., Bransby, E. R., and Hartley, H. O.: Effect of Topical Applications of Potassium Fluoride and of the Ingestion of Tablets Containing Sodium Fluoride on the Incidence of Dental Caries, *Brit. D. J.* **86**: 263, 1949.
7. Muhler, J. C., Boyd, T. M., and Van Huysen, G.: Effect of Fluorides and Other Compounds on the Solubility of Enamel, Dentin and Tricalcium Phosphate in Dilute Acids, *J. D. Res.* **29**: 182, 1950.
8. Muhler, J. C., and Day, H. G.: Relation of pH to the Effectiveness of Sodium Fluoride and Stannous Fluoride in Decreasing Enamel Solubility, *J. D. Res.* **31**: 102, 1952.
9. Muhler, J. C., and Weddle, D. A.: Fluorine Storage in Rats Receiving Different Fluorides in the Drinking Water at Varying pH, *J. D. Res.* **34**: 895, 1955.
10. Lazansky, J. P.: The Effect on Hamster Caries of Toothbrushing with Various Chemicals, *J. D. Res.* **26**: 446, 1947.
11. Muhler, J. C., Nebergall, W. H., and Day, H. G.: Comparison of Different Preparations of Stannous Fluoride With Sodium Fluoride in the Prevention of Dental Caries in Rats, *J. A. D. A.* **46**: 290, 1953.
12. Howell, C. L., Gish, C., Smiley, R., and Muhler, J. C.: The Effect of Topically Applied Stannous Fluoride on the Dental Caries Experience in Children, *J. A. D. A.* **50**: 14, 1955.

RELATIVE CARIES-INHIBITING VALUE OF TOPICALLY APPLIED SODIUM SILICOFLUORIDE AND SODIUM FLUORIDE

Final Report on a Two-Year Study at Mound, Minnesota

JOHN K. PETERSON, D.D.S., M.P.H., AND WM. A. JORDAN, D.D.S., M.P.H.

Minnesota Department of Health, Minneapolis, Minn.

A SATURATED (0.9 per cent) sodium silicofluoride solution has been reported more effective than sodium fluoride in vitro in making tooth enamel resistant to acids.[1] In April, 1953, a clinical study was begun in the Mound, Minnesota, schools to measure the possible value of topically applied sodium silicofluoride as a caries-preventive measure. Subsequently, a preliminary report[2] was published giving the study plans and DMF prevalence of the children involved in the study at the time of the initial examination, and a first-year report[3] showing caries incidence for the first year after treatment with the 2 solutions. This is the final report of the two-year study.

PROCEDURE

A total of 610, aged 8 to 14, children in grades 3 through 7 received treatments in 1953 in the 3 different projects of the program. Each received a detailed dental examination (with the use of a portable dental chair, Burton light, explorer, and an anterior and 2 posterior bite-wing x-rays), a prophylaxis, and a varying number of 0.9 per cent sodium silicofluoride and 2 per cent sodium fluoride topical treatments, depending on which of the three projects they were in.

Children in Project I received 4 treatments of sodium silicofluoride on one side of the mouth and 4 of sodium fluoride on the other. A clerk designated, by marking alternately, which side of the mouth was to be treated with each solution. Each child carried to the operator a slip on which this information was marked. Children in Project II received 2 sodium silicofluoride treatments on one side of the mouth, and nothing on the other side. Children in Project III received 1 treatment of sodium silicofluoride on one side and 4 treatments of sodium fluoride on the other side of the mouth.

Each treatment consisted of isolating the teeth on one side of the mouth by a cotton roll on the lower lingual and a continuous cotton roll on the upper and lower buccal, both held in place with a Garmer's cotton roll holder. The teeth were then dried thoroughly with compressed air and saturated with the

This study is sponsored by the Minnesota Department of Health, with the assistance of the University of Minnesota School of Dentistry, The Minnesota Dental Foundation, and the Health Council, P.T.A., and school personnel of Mound, Minn.

Received for publication Aug. 10, 1955.

designated solution. A mouth prop was used on the buccal to hold the cotton roll above the upper teeth during the 4-minute treatment. The multiple-chair technic developed by the U.S. Public Health Service was used. Most treatments were completed in 4 consecutive days, although a few children had skipped days and a few had more than 1 treatment in 1 day.

The pupils in each project were picked for convenience by dividing the number of grades into 3 groups of approximately the same size. There were originally 165 children in Project I, 249 in Project II, and 196 in Project III. At the final examination, there were 133, 199, and 156 children, respectively, in the 3 projects.

The original examinations were done by both of us, and the 1954 and 1955 examinations were done by J.K.P. Only posterior bite-wing x-rays were used for the second and third examinations. The number of sound permanent teeth and surfaces were recorded on individual charts. The form used lists the types of teeth separately and indicates conditions on the left and right sides of the mouth. The differences in DMF teeth and surfaces developed on each side of the mouth is the basis for evaluating the effectiveness of the two solutions.

TWO-YEAR RESULTS—ALL PERMANENT TEETH—PROJECT I

The children in Project I received 4 treatments of each solution on opposite sides of the mouth. During the two years after treatment, 11.9 per cent of the permanent teeth that were sound in 1953 became DMF on the sides treated with sodium silicofluoride. This proportion is 21.4 per cent more than occurred on the sides treated with sodium fluoride. On the sides treated with sodium silicofluoride, 11.7 per cent more tooth surfaces became DMF than on the sides treated with sodium fluoride. The incidence on the sodium silicofluoride sides was higher both years of the study (Table I). The differences, however, are not statistically significant.

TWO-YEAR RESULTS—ALL PERMANENT TEETH—PROJECT II

Children in Project II received 2 treatments of sodium silicofluoride on only one side of the mouth. The children in this group were in the highest grades treated (5, 6, and 7), and consequently had more erupted sound permanent teeth per child at the beginning of the study than in the other groups. Of the sound teeth 17.7 per cent became DMF on the treated sides. This was 15.7 per cent fewer than on the untreated sides. Of the surfaces 12.8 per cent less became DMF on the treated sides. Project II data are given in Table I.

TWO-YEAR RESULTS—ALL PERMANENT TEETH—PROJECT III

Children in Project III received 1 treatment of sodium silicofluoride on one side of the mouth and 4 treatments of sodium fluoride on the other. When all permanent teeth are considered, there appears to be no difference in caries incidence between the 2 treatments. These data are also given in Table I.

TABLE I
DMF INCIDENCE AFTER TREATMENT WITH SODIUM SILICOFLUORIDE AND SODIUM FLUORIDE, MOUND, MINN., 1953 TO 1955

		DMF TEETH				DMF SURFACES			
	NUMBER TREATMENTS	SOUND TEETH 1953	INCIDENCE 1953 TO 1955	PER CENT INCIDENCE 1953 TO 1955	PER CENT INCIDENCE 1953 TO 1954	SOUND SURFACES 1953	INCIDENCE 1953 TO 1955	PER CENT INCIDENCE 1953 TO 1955	PER CENT INCIDENCE 1953 TO 1954
Project I									
Sodium silicofluoride	4	725	86	11.9	6.0	4472	215	4.8	2.4
Sodium fluoride	4	735	72	9.8	4.9	4516	195	4.3	2.2
Project II									
Sodium silicofluoride	2	1718	304	17.7	9.3	10884	737	6.8	3.2
Control	0	1708	359	21.0	10.4	10830	847	7.8	3.6
Project III									
Sodium silicofluoride	1	877	129	14.7	7.8	5617	341	6.1	2.9
Sodium fluoride	4	885	131	14.8	6.9	5640	340	6.0	2.7

PAIRED POSTERIOR TEETH

Since some of the anterior teeth were unquestionably treated with both solutions, and anterior bite-wing x-rays were not used in the second and third examinations, a comparison was made using only posterior teeth. To equalize further the risk of teeth becoming DMF on each side, only sound teeth and surfaces were counted that matched (paired) with sound teeth and surfaces on the other side of the mouth. Thus, both sides had comparable teeth and surfaces vulnerable to the caries process.

Two-Year Results—Posterior Teeth—Project I.—Project I children had 4 treatments of each solution on opposite sides of their mouths. The children who were present for both the first and third examinations had 135 pairs of sound permanent posterior teeth at the first examination. In 2 years, 46 new DMF teeth developed on the sides which were treated with sodium silicofluoride, while 40 developed on the sodium fluoride treated sides. Of 1,392 sound tooth surfaces, 152 became DMF on the sodium silicofluoride sides, and 136 on the sodium fluoride sides (Table II). Even though 15 per cent more teeth and 11.8 per cent more surfaces became DMF on the sodium silicofluoride sides, the differences are not conclusive.

Two-Year Results—Posterior Teeth—Project II.—This group received 2 treatments of sodium silicofluoride on one side only. There were 571 pairs of sound permanent posterior teeth present in 1953. On the treated sides, 182 teeth became DMF, while 213 became DMF on the untreated sides. Of the 4,747 pairs of sound surfaces, 485 became DMF on the treated and 578 on the untreated sides. The reduction was 14.5 per cent in DMF teeth and 16.1 per cent in DMF surfaces on the sides treated. Statistically, the reduction in DMF teeth is of border-line significance. It could occur by chance less than

5 times in a hundred (K = 1.95). The reduction in DMF surfaces is significant and could occur by chance less than 3 times in a thousand (K = 3.1). The data for Project II are given in Table II.

Two-Year Results—Posterior Teeth—Project III.—There were 162 pairs of sound permanent posterior teeth present in 1953. One treatment of sodium silicofluoride and 4 of sodium fluoride were given on opposite sides of the mouth. Again, more teeth became DMF on the sodium silicofluoride sides (63), compared with 53 on the sides treated with sodium fluoride (Table II).

Of 1,751 surfaces, 209 became DMF on the sodium silicofluoride sides, compared with 197 on the sodium fluoride sides. On the sodium silicofluoride sides, 18.9 per cent more teeth and 6.1 per cent more surfaces developed. As in Project I, more DMF teeth and surfaces developed after treatment with sodium silicofluoride than with sodium fluoride. The difference, however, is not statistically conclusive.

TABLE II
DMF INCIDENCE IN PAIRED SOUND TEETH AND SURFACES AFTER TREATMENT WITH SODIUM SILICOFLUORIDE AND SODIUM FLUORIDE, MOUND, MINN., 1953 TO 1955

			DMF TEETH			DMF SURFACES			
	NUMBER TREATMENTS	SOUND PAIRED TEETH 1953	INCIDENCE 1953 TO 1955	PER CENT INCIDENCE 1953 TO 1955	PER CENT INCIDENCE 1953 TO 1954	SOUND PAIRED SURFACES 1953	INCIDENCE 1953 TO 1955	PER CENT INCIDENCE 1953 TO 1955	PER CENT INCIDENCE 1953 TO 1954
Project I									
Sodium silicofluoride	4	135	46	34.1	18.3	1392	152	10.9	5.0
Sodium fluoride	4	135	40	29.6	12.7	1392	136	9.8	4.9
Project II									
Sodium silicofluoride	2	571	182	31.9	15.5	4747	485	10.2	4.4
Control	0	571	213	37.3	17.5	4747	578	12.2	5.4
Project III									
Sodium silicofluoride	1	162	63	38.9	19.9	1751	209	11.9	5.6
Sodium fluoride	4	162	53	32.7	13.4	1751	197	11.3	4.4

SUMMARY AND CONCLUSIONS

A total of 610 children divided into 3 groups were given 1, 2, or 4 treatments of 0.9 per cent sodium silicofluoride on one side of the mouth and 4 treatments of 2 per cent sodium fluoride, or nothing, on the opposite side.

The caries incidence in the second year of the study corroborates the results after 1 year. Two topical treatments of 0.9 per cent sodium silicofluoride probably reduced caries incidence by about 15 per cent in the 2 years of the study. However, when matched against 4 treatments of sodium fluoride, more DMF teeth and surfaces developed with both 1 and 4 treatments of

sodium silicofluoride. While the superiority of sodium fluoride was not statistically significant, it should at least be safe to assume that sodium silicofluoride is not more effective than sodium fluoride as a topical treatment in the prevention of dental caries.

We acknowledge with thanks the assistance of Lila R. Elveback, Department of Biostatistics, School of Public Health, University of Minnesota, for consultation in the statistical analyses included in this report; and James F. Lewis, D.D.S., regional consultant of the Public Health Service, for his invaluable assistance in the treatment phase of the study.

REFERENCES

1. Massler, Maury: Is Sodium Fluoride Best for Topical Application? *J. Dent. Child.* 21: 14, 1954.
2. Peterson, J. K., Jordan, Wm. A., and Lewis, James F.: A Topical Sodium Silicofluoride and Sodium Fluoride Study; A Preliminary Report, *North-West Dentistry* 32: 183, 1953.
3. Peterson, John K., and Jordan, Wm. A.: Relative Caries-Inhibiting Value of Sodium Silicofluoride and Sodium Fluoride: First Year Report, *J. D. Res.* 35: 54, 1956.

THE INHIBITORY EFFECT OF SODIUM N-LAUROYL SARCOSINATE ON ORAL LACTOBACILLI WHEN GROWN ON VARIOUS MEDIA

I. L. SHKLAIR, Ph.D., K. C. HOERMAN, D.D.S., M.S., AND
H. R. ENGLANDER, D.D.S., M.P.H.

The Dental Research Facility, United States Naval Training Center, Great Lakes, Ill.

CATIONIC and anionic detergents (surface tension depressants) are known to exhibit various degrees of antimicrobial activity. The cationic compounds, based on the quaternary ammonium structure, for example, Zephiran chloride, have been widely used for sterilization. The anionic detergents have been reported to selectively inhibit the metabolism of gram-positive microorganisms.[1-4]

Fosdick, Calandra, Blackwell and Burrill,[5] reported that sodium N-lauroyl sarcosinate (SLS), an anionic detergent, inhibited enzymatic glycolysis in dental plaque. A subsequent report by Carbon, Blackwell, Calandra, and Fosdick[6] stated that SLS strongly inhibits preliminary phosphorylation, particularly the hexokinase reaction. There were also indications that enolase inhibition, and possibly other enzymatic phases of the glycolytic scheme, were similarly affected.

The anionic detergent, SLS, is currently being utilized in some dentifrices in an effort to prevent tooth decay. Its use is based upon the assumed prevention of acid production in the dental plaque subsequent to carbohydrate degradation.

Lactobacilli are commonly used as indices of caries activity. These organisms can be grown on various media. This study was undertaken to determine the influence of SLS upon the growth of lactobacilli when cultured on different media.

PROCEDURES AND RESULTS

Filter paper disc and tube dilution methods were employed to determine the inhibitory effect of SLS on the lactobacilli strains.

Procedure I. The Filter Paper Disc Method.—Fifteen strains of homofermentative and heterofermentative lactobacilli were isolated from the whole salivas of caries-active individuals. The organisms were grown in Rogosa broth for 24 to 48 hours. One-tenth milliliter of this suspension was seeded over the following agar plates: (1) Tomato juice agar containing sodium azide (NaN_3) 1:10,000, (2) tomato juice agar without sodium azide 1:10,000, and (3) Rogosa agar without sodium azide.

The opinions or assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Department of the Navy or the Naval Service at large.

Received for publication Sept. 21, 1955.

Filter paper discs (Schleicher and Schuell, No. 740 E), 12.5 mm. in diameter, were moistened with various concentrations of SLS (1.0, 2.5, 5.0, and 10.0 mg./ml.) and placed on the various agar plates. These plates were then incubated at 37.5° C. and the zones of inhibition measured after 24 to 48 hours.

Results: SLS produced correspondingly larger zones of inhibition with increasing concentrations. At equal concentrations, larger zones of inhibition occurred on the tomato juice agar than on Rogosa agar. Tomato juice agar with sodium azide exhibited greater zones of inhibition than tomato juice agar alone. Conversely, no synergistic effect was noted between SLS and sodium azide in Rogosa agar (Table I). In order for this synergism to occur on tomato juice agar, the plate must be used within 3 days subsequent to the addition of the sodium azide.

TABLE I
THE EFFECT OF SODIUM N-LAUROYL SARCOSINATE ON 15 STRAINS OF ORAL LACTOBACILLI

DILUTION MG./ML. SLS	ROGOSA AGAR	ROGOSA AGAR SODIUM AZIDE 1:10,000	TOMATO JUICE AGAR	TOMATO JUICE AGAR SODIUM AZIDE 1:10,000
1.0	16*	16	22	35
2.5	19	19	25	43
5.0	23	23	37	52
10.0	26	26	41	57

*Average zones of inhibition in millimeters.

In this procedure, penicillin in concentrations of 0.1 to 20 units per milliliter was substituted for SLS in order to compare inhibitory effects. The variations in the media did not affect the inhibitory action of penicillin as they did with SLS. The degree of inhibition varied only, in the case of penicillin, with the strains tested.

Procedure II. Tube Dilution Method.—The organisms mentioned previously were tested in Rogosa and tomato juice broth preparations with and without sodium azide. Ten tube serial dilutions of SLS ranging from 1 to 0.0039 mg. per milliliter were prepared. The tubes were inoculated and incubated for 48 hours. The bacteriostatic and bactericidal action of SLS was determined by subculturing from broth tubes exhibiting no growth.

Results: A greater degree of inhibition by SLS was noted in the tomato juice broth than in the Rogosa broth. This result paralleled those observed in the agar plates, however, the addition of sodium azide to the tomato juice broth did not increase the inhibitory effect of SLS as it did in the tomato juice agar plates (Table II).

Growth was evident after subculturing from the tube showing inhibition at the lowest concentration of SLS. This indicated a bacteriostatic action. The remaining tubes containing higher concentrations of SLS showed no growth upon subculturing thus indicating a bactericidal action. Some strains of

lactobacilli were inhibited at 0.015 mg. per milliliter of SLS. All were inhibited at 0.25 mg. per milliliter, and all showed bactericidal action at 0.5 mg. per milliliter.

TABLE II

THE INHIBITION OF L. CASEI (VAR. RHAMNOSES) BY SLS (TUBE DILUTION METHOD)

MEDIA	CONCENTRATION OF SLS (MG./ML.)									
	1	0.5	0.25	0.125	0.0625	0.0312	0.0156	0.0078	0.0039	CONTROL
Tomato broth	–	–	0	–	–	0	+	+	+	+
Tomato broth sodium azide 1:10,000	–	–	0	–	–	0	+	+	+	+
Rogosa broth	–	–	–	+	+	+	+	+	+	+
Rogosa broth sodium azide 1:10,000	–	–	–	+	+	+	+	+	+	+

+growth in 48 hours.
– no growth in 48 hours.
0 growth after subculture.

SUMMARY AND CONCLUSIONS

There appeared to be a synergistic effect between SLS and sodium azide on the inhibition of lactobacilli when grown on tomato juice agar. This effect was absent on Rogosa agar. This synergism was absent in all tube dilution tests. There were, however, greater degrees of inhibition by SLS in the tomato juice broth and agar than in Rogosa broth and agar.

The results relative to inhibition of lactobacilli on tomato juice agar, using the filter paper disc method, were in agreement with those of Rosenthal and Abriss.[7] They employed only one strain of *Lactobacillus acidophilus* and concluded that SLS acted as a bacteriostatic agent. We have found that SLS, depending upon the concentrations employed, can act either as a bacteriostatic or bactericidal agent.

Rogosa medium is more selective and nutritionally complete than the tomato juice agar or broth. Therefore, the degree of inhibition of lactobacilli by SLS appears to be related inversely to the nutritional value of the media. Penicillin inhibition of lactobacilli was not related to the medium employed.

REFERENCES

1. Baker, Z., Harrison, R. W. and Miller, B. F.: Action of Synthetic Detergents on the Metabolism of Bacteria, *J. Exper. Med.* 73: 249, 1941.
2. McCullough, E. C.: The Efficiency of Soaps and Other Disinfectants in Destroying Mastitis Streptococci, *Am. J. Vet. Res.* 1: 18, 1940.
3. McCullough, E. C., and Fuller, S. A.: The Relative Efficiency of Disinfectants in Killing Erysypllothrix Rhusiopathiae, *Am. J. Vet. Res.* 2: 77, 1941.
4. Schwartz, A. M., and Perry, J. W.: Surface Active Agents, New York, 1949, Interscience Publishers, Inc., p. 455.
5. Fosdick, L. S., Calandra, J. C., Blackwell, R. Q., and Burrill, J. H.: A New Approach to the Problem of Dental Caries Control, *J. D. Res.* 32: 486, 1953.
6. Carbon, J. A., Blackwell, R. Q., Calandra, J. C., and Fosdick, L. S.: The Inhibition of Anaerobic Glycolysis on Sodium N-Lauroyl Sarcosinate, *Arch. Biochem.* 55: 356, 1955.
7. Rosenthal, M. W., and Abriss, A.: Some Laboratory Observations on the Chemical, Bacteriological and Enzymatic Properties of Sodium N-Lauroyl Sarcosinate, *J. Dent. Child.* 21: 194, 1954.

A CLINICAL STUDY OF BLOOD PRESSURE RESPONSES TO EPINEPHRINE-CONTAINING LOCAL ANESTHETIC SOLUTIONS

FRANK M. MC CARTHY, M.D., D.D.S., M.S.

Los Angeles, Calif.

THE possible hemodynamic pressor response initiated by epinephrine has received considerable attention from both the dental and medical professions. This is of particular importance when the patient presenting for local anesthesia is suffering from heart disease or hypertension. The classical sympathomimetic effect of epinephrine is peripheral vasoconstriction characterized by elevated arterial blood pressure, increased heart rate, and increased cardiac output; effects feared in the patient with cardiovascular disease. They may precipitate anginal syndrome, myocardial infarction, cardiac decompensation, or cerebral hemorrhage. However, with small amounts of epinephrine, only relaxing effects are demonstrated. Peripheral vasodilatation occurs rather than the vasoconstriction resulting from higher doses.[1]

In addition, the hypersensitive, excitable patient who labels himself "allergic" to local anesthetic solutions may exhibit symptoms of increased excitement, palpitation, tremor, and sweating when local anesthesia is administered. Epinephrine in the anesthetic solution is frequently indicted for this reaction.

If anesthesia without epinephrine is used for cardiac patients and for those who exhibit so-called hypersensitivity, the clinician is faced with the possibility of anesthetic failures and lack of anesthetic depth and duration. This inadequate anesthesia results in pain which may trigger the release of substantial amounts of epinephrine from the adrenal medulla, and the clinician has thus caused exactly the condition which he wished to avoid, excitement, increased blood pressure, and increased strain on the heart.

For purposes of comparison, the epinephrine content per cubic centimeter of commonly used solutions follows:

Epinephrine 1:1,000	1 mg. per c.c.
Epinephrine 1:50,000	.02 mg. per c.c.
Epinephrine 1:100,000	.01 mg. per c.c.

The commonly employed dental preparations (1:50,000 or 1:100,000) contain 1/50 and 1/100, respectively, the amount of epinephrine in the commonly employed medical preparation (1:1,000). It is stated that 0.5 mg. of epinephrine is the effective dose for positive or pressor effects in man when injected subcutaneously.[2]

Presented in part at the Thirty-second General Meeting of the International Association for Dental Research, French Lick, Indiana, March 19, 1954 (*J. D. Res.* 33: 674, 1954).

This study was performed in partial fulfillment of the requirements for the degree of Master of Science in Oral Surgery at Georgetown University and was supported by a post-doctorate research fellowship from the National Institutes of Health and by a grant-in-aid from Astra Pharmaceutical Products, Inc., Worcester, Mass.

Received for publication Sept. 10, 1955.

While considerable animal and clinical experimentation has been carried out, it is as yet impossible to draw accurate conclusions regarding the possibility of pressor effects from epinephrine in dental local anesthetic solutions.

Using dogs from 6 to 10 kilograms in weight, Luckhardt and Koppanyi[3] subcutaneously administered an average of 0.54 c.c. of 1:1,000 epinephrine per kilogram of body weight. They found that massage of the injected site hours later produced a pressor effect. There was no response whatever during profound anesthesia. Pickering, Steinmeyer and Luckhardt performed submucous injections at the mucobuccal fold in dogs.[4] A volume of 2.0 c.c. of 2 per cent procaine solution ranging from 1:10,000 to 1:30,000 epinephrine content was used. There were blood pressure rises of 10 to 40 mm. Hg, much greater than those recorded from similar injections in the subcutaneous tissue. Following Koppanyi's technic, Pickering found that light massage of the injection site produced a greater pressor response. Nakauchi later repeated this finding on dogs and also reported that pressor effects were obtained with difficulty in all regions of the mouth except the site of the palatine injection.[5]

Clinical experimentation introduces difficult problems of interpretation. In his review of local anesthetic reactions, Tainter stated that, "One of the first problems is whether the reaction is due to the local anesthetic, or rather to the psychological influences acting on the patient before and during the dental procedure."[6] Tainter claimed that 90 per cent of the clinical reactions seen are due to psychic factors.[7] Adding to the difficulties of clinical interpretation is the fact that one must often depend entirely upon subjective symptoms which are difficult to evaluate.

Clinical studies employing epinephrine-containing local anesthetic solutions have been reported by Tainter and Winter,[8] Tainter, Throndson, and Moose,[9] Borg,[10] Miller and Stuart,[11] and Follmar.[12] They reported that the epinephrine had little or no pressor effect. In a review of the dental and medical literature, Dick found no fatalities attributed to the use of epinephrine in dental local anesthetic solutions.[13] He stated that over 30 million injections of local anesthetic solutions containing epinephrine are given yearly in the United States for dental purposes.

Epinephrine, as used in this experiment and as generally referred to, is actually a mixture of l-epinephrine and l-norepinephrine (l-arterenol or Levophed). Assay has indicated that norepinephrine constitutes from 16 to 36 per cent of natural U.S.P. epinephrine.[14]

The present study was initiated to investigate the possible pressor effects that may occur following the injection of local anesthetic agents with and without epinephrine, to investigate possible differences in response related to various sites of intraoral injection, and to investigate possible psychosomatic pressor responses caused by fear of the injection. Every effort was made to keep the experiment as closely controlled as possible and to develop a dependable method of analysis. It was designed as a pilot study for further investigation on patients with cardiac disease and hypertension.

METHODS

Subjects consisted of 40 male, senior dental students from 21 to 32 years of age. They were in currently good health and had no history of cardiovascular disease or of past illnesses which could conceivably affect the results. Choice of subjects was made in the order of volunteering, and they were rejected only if they did not meet the above criteria or if their preliminary blood pressure determinations were consistently above 150/100 mm. Hg. Readings consistently above 150/100 mm. Hg were assumed to indicate clinical hypertension. No effort was made to eliminate those subjects who demonstrated unusually labile blood pressures.

Prior to actual testing, and with the subjects aware that no injection was to be performed during that appointment, they were examined in groups of 4 for preliminary blood pressure and pulse rate determinations. Four values were taken over a 30-minute period. The subjects were instructed to sit quietly during that time. They were then divided into 2 groups of 20 by choosing alternate names in the order of volunteering. Right submucous posterior palatine injections of 0.5 c.c. of test solution were administered to one group and left submucous maxillary mucobuccal fold injections of 1.0 c.c. at the second premolar region were administered to the second group.

Six treatments were performed on each subject. They are outlined and have been numbered for convenience: Treatment 1—4 per cent procaine plain, Treatment 2—4 per cent procaine with epinephrine 1:50,000, Treatment 3—2 per cent Xylocaine plain, Treatment 4—2 per cent Xylocaine with epinephrine 1:100,000, Treatment 5—isotonic saline, and Treatment 6—pseudoinjection.

Inasmuch as clinical application was given as much consideration as possible, 4 per cent procaine with and without epinephrine 1:50,000 was used because it more closely approximated the Xylocaine solutions in anesthetic potency. The first 5 treatments or injections were administered in the usual manner. The injection site was not massaged. For Treatment 6, or pseudoinjection, the usual routine was followed, the syringe was placed in position in the subject's mouth, but the needle did not touch the mucosa. The treatments were randomized so that no subject knew what he would receive at any appointment. The only change made was to substitute any pseudoinjection falling on the last appointment for one of the earlier treatments. This was done in rotation to maintain uniform distribution of all other treatments. No operative dentistry or surgery was permitted after any treatment.

Lock-type 5 c.c. syringes with ½-inch 27-gauge needles were used routinely. Blood pressure was determined by the auscultatory method over the left brachial artery with an aneroid sphygmomanometer. Pulse rate per minute was determined by counting the pulse at the left radial artery for 15 seconds and multiplying by 4. While somewhat lacking in accuracy, it was not possible to allocate more time for this value. It was realized that significant changes in pulse rate would be apparent. An interval timer, calibrated in seconds and minutes, was used.

An attempt was made to duplicate as much as possible the usual routine of a dental office prior to actual commencement of test. The treatment room was quiet and not subject to interruptions. After waiting in a private room for up to 10 minutes, the subject was brought into the treatment room where he rinsed his mouth with an antiseptic solution. Talking before the first blood pressure and pulse determinations were made was not discouraged and was left up to the subject. Talking, coughing, or unnecessary movement after the experiment had begun was discouraged. After rinsing, the subject then sat in the dental chair, the sphygmomanometer cuff was adjusted, and blood pressure and pulse determinations were made and called the zero readings. Immediately thereafter the injection or pseudoinjection was made. The blood pressure and pulse rate were then noted at 1-minute intervals for 10 minutes.

The operator and subject were alone in the treatment room at all times. The operator performed all of the treatment steps and determinations himself. He also was aware of the identity of the various solutions. No attempt was made to evaluate resultant anesthesia. Subjects were instructed to report any symptoms of reaction following the end of each treatment.

RESULTS

I. *Preliminary Blood Pressure and Pulse Rate Determinations.*—The means of preliminary blood pressure and pulse rate determinations were determined by 4 readings taken over a 30-minute period. The means of the preliminary buccal site means are: B.P. 120/81, pulse 71. The means of the preliminary palatine site means are: B.P. 118/75, pulse 71.

II. *Comparison of Blood Pressure and Pulse Rate Responses for All Treatments and Both Sites.*—Blood pressure and pulse rate deviations noted by comparing preliminary means with zero time means were similar for all treatments at both sites. The average zero time means show an increase of 8 mm. systolic pressure, a 10.5 mm. diastolic fall, and an increase of 2 in pulse rate as compared to the preliminary means. Comparison of postinjection pressure and pulse means for Treatments 1, 3, 5, and 6 at the buccal site and Treatments 1, 2, 3, 4, and 6 at the palatine site shows that results were similar. There was an average fall of 6 mm. systolic by 1 minute, and this gradually continued until a maximum fall of 12 mm. was reached at 10 minutes. There was little fluctuation of diastolic pressure or of pulse rate. Considering Treatments 2 and 4 at the buccal site and Treatment 5 at the palatine site, the systolic pressure and pulse rate postinjection means were similar to those of all other treatments. However, the diastolic means of Treatments 2 and 4 at the buccal site indicated a gradual postinjection fall reaching a low of minus 7 mm. at 10 minutes. The diastolic means of Treatment 5 at the palatine site indicated a fairly rapid postinjection increase to a high of plus 6 mm. at 7 minutes and a subsequent drop to plus 4 mm. at 10 minutes. All zero time and postinjection blood pressure and pulse rate means for both sites are outlined in Table I.

TABLE I
POSTINJECTION MEAN READINGS, PRESSURE AND PULSE

BUCCAL SITE

TIME (MINUTES)		0	1	2	3	4	5	6	7	8	9	10
T-1	S	127	121	119	119	118	117	116	116	116	115	115
	D	70	69	70	70	70	71	71	71	71	70	70
	P	73	73	73	74	75	75	74	74	74	75	74
T-2	S	127	121	118	117	116	115	116	115	114	115	114
	D	70	69	68	66	65	65	64	64	63	63	62
	P	72	70	72	72	73	73	72	73	73	73	72
T-3	S	127	122	118	118	116	115	116	114	116	115	113
	D	69	69	69	70	70	70	71	70	71	71	70
	P	72	70	72	71	72	72	74	73	73	72	73
T-4	S	128	121	120	119	118	117	116	115	116	116	116
	D	72	70	70	70	69	68	67	67	67	67	66
	P	74	71	73	74	74	75	75	75	75	75	75
T-5	S	125	120	118	117	116	115	115	114	114	114	113
	D	68	68	69	70	69	69	70	69	70	70	69
	P	74	68	69	71	71	72	71	71	72	71	71
T-6	S	128	122	121	119	119	118	116	115	116	116	116
	D	70	71	71	70	71	70	71	70	71	71	70
	P	73	72	72	73	74	73	74	74	74	74	74

PALATINE SITE

TIME (MINUTES)		0	1	2	3	4	5	6	7	8	9	10
T-1	S	126	121	119	117	115	115	115	113	114	114	115
	D	64	64	65	66	65	65	66	66	66	66	66
	P	72	69	70	71	71	70	70	70	71	71	70
T-2	S	126	122	120	118	117	116	116	116	116	115	115
	D	65	64	64	63	63	63	63	63	64	64	62
	P	72	70	71	71	72	72	73	72	72	72	71
T-3	S	129	122	120	118	116	115	115	115	114	113	114
	D	66	65	65	65	64	65	66	65	65	65	65
	P	75	71	72	73	72	73	72	72	72	71	72
T-4	S	128	122	121	119	117	116	116	115	116	116	115
	D	70	67	67	67	68	67	67	67	67	67	66
	P	73	71	74	74	75	75	74	73	73	74	73
T-5	S	127	121	118	117	115	115	114	115	113	114	113
	D	63	65	66	66	67	67	68	69	69	68	67
	P	73	70	69	69	71	71	71	72	71	71	72
T-6	S	126	121	119	117	117	116	116	115	115	115	115
	D	65	65	64	65	65	66	67	67	67	67	66
	P	75	73	75	76	75	75	76	76	75	74	74

III. *Training Effect.*—While the treatments were randomized to eliminate deviations from a possible training effect, it is interesting to note that no training effect occurred. Means of blood pressure determinations at the preliminary examination and at each of the 6 treatment appointments were analyzed according to the pressure (systolic or diastolic) and the site of injection. The order of magnitude of pressor responses compared to chronological sequence of blood pressure determinations is summarized in Table II. The vertical columns start with the preliminary magnitude value and continue to

the right in chronological sequence of appointments from 1 to 6. Treatments are ignored. Magnitude values of 7 and 1 represent the high and low, respectively, for each analysis. The values follow no significant pattern in relation to the appointments. As demonstrated previously, preliminary values for systolic pressure (magnitude 7) are higher than those for any of the succeeding appointments, and preliminary values for diastolic pressure (magnitude 1) are lower than those for any of the succeeding appointments.

TABLE II
ORDER OF MAGNITUDE OF PRESSOR RESPONSE COMPARED TO CHRONOLOGICAL SEQUENCE OF BLOOD PRESSURE DETERMINATIONS

	PRELIM.	APT. 1	APT. 2	APT. 3	APT. 4	APT. 5	APT. 6
Systolic, buccal	7	5	2	3	4	6	1
Systolic, palatine	7	5	1	3	4	2	6
Diastolic, buccal	1	6	5	7	3	4	2
Diastolic, palatine	1	5	6	4	7	2	3

IV. *Analysis of Variance.*—In setting up the analysis of variance, an index number was calculated for both the systolic and diastolic pressures of each of the 240 treatments. This value was obtained by first dividing the numeral 1 by the zero time blood pressure reading (either systolic or diastolic). The quotient was then multiplied by each of the 10 postinjection blood pressure determinations and the sum of the resultant 10 answers was calculated. This sum multiplied by 100 gave the index number. Taking the systolic pressure responses of Patient 1, buccal site, Treatment 2 as an example, the numeral 1 is divided by the zero systolic pressure of 128. The quotient of .0078125 is multiplied by the one-minute postinjection systolic pressure of 126, and the same multiplication is repeated using the remaining 9 postinjection systolic pressures as multipliers. The sum of the answers is 9.35937. Multiplied by 100, the resultant index number is 935.94. An index number of 1,000 indicates that the postinjection pressures have remained constant as compared to the zero time pressure. A higher number indicates a comparative increase, while a lower number indicates a comparative decrease.

Analyzed items were treatments, patients, and site. Summaries of the analyses for systolic pressure and for diastolic pressure are outlined in Tables III and IV, respectively.

TABLE III
ANALYSIS OF VARIANCE
SUMMARY FOR SYSTOLIC PRESSURE

ITEM	D.F.	S.S.	M.S.	F	P*
Treatment	5	6,632.2666	1,326.4533	.8436	n.s.
Patients	39	155,383.4264	3,984.1904	2.5338	$<.01$
Site	1	98.0737	98.0737	.0624	n.s.
Error	194	305,037.3644	1,572.3575		
Total	239	467,151.1311			

*P—Probability for observed variations to be due to chance.

TABLE IV
ANALYSIS OF VARIANCE
SUMMARY FOR DIASTOLIC PRESSURE

ITEM	D.F.	S.S.	M.S.	F	P*
Treatment	5	193,536.5851	38,707.3170	7.7936	<.01
Patients	39	646,524.6086	16,577.5530	3.3377	<.01
Site	1	46,516.4648	46,516.4648	9.3657	<.01
Error	194	963,532.3626	4,966.6616		
Total	239	1,850,180.0211			

*P—Probability for observed variations to be due to chance.

Considering the analysis of variance for systolic pressure, the F value for treatment and for site is not significant. This is understandable inasmuch as observation of the gross results indicates close uniformity of systolic response to all treatments and sites. The F value for patients indicates a probability of less than 1 per cent for observed variations to be due to chance.

Considering the analysis of variance for diastolic pressure, the F value indicates a probability of less than 1 per cent for observed variations to be due to chance for all items, treatments, patients, and site. The variations of Treatments 2 and 4 at the buccal site and Treatment 5 at the palatine site will be discussed later. It was not possible to perform an analysis on volume injected because of its association with site (buccal site—1.0 c.c., palatine site—0.5 c.c.). Thus, it is possible that an observed variation for site may also involve volume. This point will be differentiated during later discussion.

DISCUSSION

I. *Preliminary Blood Pressure and Pulse Rate Determinations.*—While the normal for both blood pressure and pulse rate is subject to wide individual variations, the means of this experiment approximate average values for the young adult male. As given by Best and Taylor,[15] these values are: blood pressure—120/80, mean pressure—100, pulse pressure—40, and pulse rate—70. Mean preliminary values for all subjects in this study are: blood pressure—119/78, mean pressure—98.5, pulse pressure—41, and pulse rate—71.

II. *Psychosomatic Response Related to Fear of the Injection.*—Emotional excitement tends to increase arterial blood pressure, particularly the systolic phase, and the heart rate is also increased.[15] Mean preliminary values compared to mean zero time values for all subjects in this experiment indicate an 8 mm. systolic increase, a 10.5 mm. diastolic decrease, and an increase of 2 in pulse rate immediately before the injection or pseudoinjection. It is felt that the increased heart rate was a direct result of sympathetic stimulation, that the increase in systolic pressure was the result of increased cardiac output, and that the decrease in diastolic pressure was the result of peripheral vasodilatation with decreased peripheral resistance (negative sympathetic effect). One minute after the injection, there was an average fall of 6 mm. in the systolic pressure for all treatments, and this response had increased to an average fall of 12 mm. by the 10-minute determination. Pulse rate remained relatively constant throughout each treatment. Diastolic pressure, with 3 exceptions, also remained relatively constant throughout the treatments. The

above data demonstrate a definite cardiovascular response immediately before and after the injection itself, such response not being related to the test solution or to the injection site.

III. *Blood Pressure Responses to Local Anesthetic Solutions With and Without Epinephrine, and to Isotonic Saline and a Pseudoinjection.*—As indicated by the statistical analysis, no pressure or pulse rate response may be attributed to the following treatments or to the injection sites: Treatments 1, 3, 5, and 6, buccal site; Treatments 1, 2, 3, 4, and 6, palatine site.

Treatments 2 and 4, buccal site, 4 per cent procaine with epinephrine 1:50,000 and 2 per cent Xylocaine with epinephrine 1:100,000, respectively, produced nearly identical falls in diastolic pressure. Systolic pressure and pulse rate responses were similar to all other treatments. There was a gradual postinjection decrease in diastolic pressure attaining a maximum fall of 7 mm. at 10 minutes. The mechanism for this fall is the depressor action of small amounts of epinephrine. This action has long been recognized in animal experimentation.[16] It results in peripheral vasodilatation and a fall in arterial blood pressure. The author feels that this effect was not repeated using the same solutions at the palatine site for 2 reasons: (1) because the volume for the palatine injection was half that for the buccal injection, and (2) because the greater pain attending the palatine injection may have caused a sympathetic response which masked a tendency for depression of diastolic pressure. The latter theory gains support and the volume deficiency theory is minimized when it is considered that absorption from a palatine injection is more rapid than from a buccal injection.[5]

Treatment 5, palatine site, isotonic saline, produced a diastolic pressure increase. Systolic pressure and pulse rate responses were similar to all other treatments. There was a fairly rapid diastolic increase to a high of 6 mm. at 7 minutes postinjection and a subsequent drop to a high of 4 mm. at 10 minutes. Double the volume of this same solution produced no response when injected at the buccal site. This response was due to the fact that the pain produced by the palatine injection of isotonic saline was of greater magnitude and longer duration than that produced by injection of an anesthetic solution which commences to anesthetize the area shortly after the first drop is deposited. It is felt that the pain triggered a positive sympathetic effect which resulted in peripheral vasoconstriction and increased peripheral resistance. The palatine injection is more painful than the buccal injection because the submucosa at the mucobuccal fold consists of loose connective tissue while the palatine submucosa is firm, inelastic, and closely attached to sensitive periosteum. The pain factor was obvious during actual testing. While subjects were not asked to report pain, a substantial number complained about palatine injections after the termination of the treatment. The greatest number of complaints concerned saline injections. The operator also noticed that the palatine injection of saline frequently produced phonation and movement during actual deposition of solution.

IV. *Lack of Training Effect.*—It was expected that zero time pressure and pulse determinations in later treatments might fluctuate in comparison to earlier treatments, but this was not the case. While most subjects receiving palatine injections expressed distate for repetition, their pressure and pulse responses remained essentially the same.

V. *Miscellaneous Results.*—Results not reported elsewhere include the fact that no untoward symptoms such as tremor, palpitation, or nervousness were reported following any of the treatments. Other than the previously mentioned pain reactions, the operator noticed no signs of so-called epinephrine reaction such as excitement or sweating. Although the subjects were not asked to report resultant anesthesia or lack of same following each treatment, a number complimented the operator's technic after the pseudoinjection and several reported profound anesthesia. This psychosomatic phenomenon was more forcefully demonstrated by Follmar when he performed painless oral surgery on 5 patients out of a total of 50 who received saline control injections in a clinical study.[12]

VI. *Clinical Application of the Results.*—Since psychosomatic response to the injection was so evident in this experiment, premedication should be employed routinely when administering local anesthesia to the patient with heart disease, particularly coronary artery insufficiency, and hypertension. Premedication should also be routine for the hypersensitive, prehypertensive type who exhibits symptoms of fright reaction when receiving local anesthesia.

It is possible to predict that the blood pressure response of a normal patient to the volume of anesthetic solutions used in this experiment will follow the reported patterns, although magnitude of response may be somewhat increased. The 1.0 c.c. volume used will produce adequate anesthesia for most operative procedures. Carrying this hypothesis a step further, it has been shown that Xylocaine alone produces hemodynamic depressor effects.[5] It would therefore seem possible to use 2.0 c.c. of Xylocaine with epinephrine 1:100,000 and expect relatively the same response as that demonstrated for 1.0 c.c. of procaine with epinephrine 1:50,000. The use of 2.0 c.c. of this anesthetic solution would produce adequate anesthesia for practically any simple exodontia problem.

It would be of interest to repeat portions of this experiment using larger volumes of anesthetic solutions containing epinephrine. It is probable that the hemodynamic depressor effect of epinephrine, as demonstrated in this study, would be canceled by somewhat larger doses and that a pressor effect would be demonstrated by increasing the dose even more. Such results would parallel the classic work of Hoskins and McClure who first demonstrated this sequence in dogs.[16]

Since it is known that the hypertensive individual is a hyperreactor to epinephrine,[17] and since cardiac and hypersensitive patients may fall into this same category, the results of this experiment cannot be applied to these individuals. It is felt that small amounts of epinephrine-containing local anesthetic solutions will not produce significant pressor responses in these patients, but it is emphasized that conclusions must wait for a clinical analysis.

SUMMARY

1. A clinical study of blood pressure responses to local anesthetic solutions, with and without epinephrine, and to isotonic saline and a pseudoinjection, was carried out. A method of testing and of analyzing responses was devised.

2. Preliminary blood pressure and pulse rate determinations were taken which closely approximate reported normal values.

3. Psychosomatic hemodynamic pressor and heart rate responses caused by fear of the injection were shown, thus demonstrating the need for routine premedication of the hypersensitive patient and the patient with cardiovascular disease.

4. A mild hemodynamic pressor response was shown associated with the palatine injection of isotonic saline. No pressor response occurred with the other treatments.

5. It was demonstrated that small amounts of epinephrine cause a hemodynamic depressor effect.

6. No fright reactions occurred with any of the treatments, and no training effect occurred.

REFERENCES

1. White, J. C., Smithwick, R. H., and Simeone, F. A.: The Autonomic Nervous System, New York, 1952, The Macmillan Co., p. 98.
2. *Ibid.*
3. Luckhardt, A. B., and Koppanyi, T.: Studies on the Hemodynamic Action of Subcutaneously Injected Epinephrine. I. Some Conditions Under Which Hypodermically Administered Epinephrine Gives a Pressor Effect, *Am. J. Physiol.* **81**: 436, 1927.
4. Pickering, P. P., Steinmeyer, H. P., and Luckhardt, A. B.: Hemodynamic Effects of Subcutaneous, Submucous, and Subgingival Injections of Procaine-Epinephrine Solutions, *Proc. Soc. Exper. Biol. & Med.* **37**: 729, 1938.
5. Nakauchi, M. M.: An Experimental Study of Hemodynamic Pressor Responses to Epinephrine-Containing Local Anesthetic Solutions, M.S. dissertation, Dept. of Oral Surgery, Georgetown University, January, 1952.
6. Tainter, M. L.: Prevention and Treatment of Local Anesthetic Reactions, *J. California D. A. & Nevada D. Soc.* **28**: 324, 1952.
7. *Idem:* Reactions to Local Anesthetics, *J. South. California D. A.* **7**: 118, 1940.
8. Tainter, M. L., and Winter, L.: Some General Considerations in Evaluating Local Anesthetic Solutions in Patients, *Anesthesiology* **5**: 470, 1944.
9. Tainter, M. L., Throndson, A. H., and Moose, S. M.: Vasoconstrictors on the Clinical Effectiveness and Toxicity of Procaine Anesthetic Solutions, *J. A. D. A.* **25**: 1321, 1938.
10. Borg, F. H.: Effect of Procaine-Epinephrine Solutions on the Pulse Rate and Blood Pressure, *J. A. D. A.* **21**: 868, 1934.
11. Miller, H. C., and Stuart, C. W.: Comparative Merits of Vaso-Constrictors in Local Anesthetic Solutions: Their Practical Application, *J. A. D. A.* **23**: 1883, 1936.
12. Follmar, K. E.: A Study of the Effect on Blood Pressure of Epinephrine When in Combination With a Two Per cent Procaine Hydrochloride Solution, Unpublished M.S. dissertation, Dept. of Oral Surgery, Northwestern University, May, 1952.
13. Dick, S. P.: Clinical Toxicity of Epinephrine Anesthesia, *Oral Surg., Oral Med., & Oral Path.* **6**: 724, 1953.
14. Goldenberg, M., Faber, M., Alston, E. J., and Chargoff, E. C.: The Determination of Arterenol in Epinephrine, *Science* **109**: 537, 1949.
15. Best, C. H., and Taylor, N. B.: The Physiological Basis of Medical Practice, Baltimore, 1945, The Williams & Wilkins Co., p. 127, 205.
16. Hoskins, R. G., and McClure, C. W.: The Adrenal Glands and Blood Pressure, *Arch. Int. Med.* **10**: 343, 1912.
17. Friedberg, C. K.: Diseases of the Heart, Philadelphia, 1950, W. B. Saunders Co., p. 857.

HISTOCHEMICAL AND ELECTRON MICROSCOPY STUDIES OF DENTIN IN TRYPTOPHAN-DEFICIENT RATS

SOL BERNICK AND L. A. BAVETTA

Department of Anatomy, School of Medicine and Department of Biochemistry and Nutrition, School of Dentistry, University of Southern California, Los Angeles, Calif.

IN A PREVIOUS investigation, Bavetta and Bernick[1] showed that the dentin of the incisors of rats fed a deficient diet for a period of 7 weeks exhibited an increased number of interglobular areas indicative of a disturbance in the process of calcification. Inasmuch as the mineral intake was not altered by the dietary deficiency, it became of interest to ascertain what factors were responsible for the observed failure of calcification in the absence of tryptophan. It was therefore decided to use a number of histochemical and electron microscopy technics in an effort to detect chemical changes not readily evident by standard histological methods and ordinary light microscope. It was felt that these methods may provide us with a possible explanation for the failure of these tissues to calcify.

MATERIALS AND METHODS

Sixty male rats of the University of Southern California strain were used in this investigation. The animals were divided into 2 groups. The experimental animals (Group 1) were placed on a tryptophan-deficient diet immediately after weaning and for periods of 3 to 26 weeks. The control group was provided with an acid hydrolysate of casein which was supplemented with 1 per cent DL tryptophan. The heads were removed at the time of sacrifice and fixed immediately in an 80 per cent alcoholic Bouin's (picric-acetic-formalin) solution for at least 4 days. The specimens were decalcified in 10 per cent nitric acid in 10 per cent formalin and washed with a saturated lithium carbonate solution. They were then dehydrated and infiltrated in the routine manner and finally embedded in 50 per cent nitrocellulose. These blocks of tissue were sectioned at 20 to 30 microns in thickness and stained by the following histochemical methods: (1) Toluidin blue for the property of metachromasia. (2) Periodic acid-Schiff reaction for the presence of acid mucopolysaccharide. (3) Gomori's alkaline phosphatase reaction.

Blocks of incisal dentin of approximately 1 to 2 sq. mm. were prepared for the electron microscopy studies by the n-butyl methacrylate embedding method. The embedded blocks were then sectioned with glass knives and micrographs prepared.

Supported by Dazian Foundation for Medical Research and Office of Naval Research, Department of Navy O.N.R. 228(05).

Received for publication Oct. 24, 1955.

It was noted in a previous investigation concerning the innervation of the dentition of the rat[3] that peptic hydrolysis of blocks of tissue removed or so altered the stainability of the collagenous elements that an unmasking of the nerves occurred. It was therefore felt that the process of peptic digestion might be utilized in an attempt to detach the protein components of the matrix from their association with the carbohydrate member of the ground substance. If the failure of calcification in the experimental animals was the result of a deficient protein synthesis, then a similar picture could be produced in the control animals by either partial or complete peptic digestion of this protein matrix. Consequently blocks of tissue from the control animals were subjected to peptic digestion at 37° C. for varying periods of time depending upon the size of the blocks. After such enzymatic treatment the blocks of tissue were prepared for sectioning in the manner just described.

OBSERVATIONS

The dentin of the incisor in the rat is composed of a fibrillar matrix in which minerals become embedded during the process of dentinogenesis. The matrix is composed of fine collagenous fibrils of 640 A. spacings and a ground substance containing a protein mucopolysaccharide complex. It is believed that the organic matrix becomes calcified in the form of globules which when fused together gives a homogenous appearance to the dentin. Fig. 1 shows a hematoxylin and triosin stained section of the dentin from a control animal. It is possible to distinguish 2 dentinal layers: an inner light pink zone, the uncalcified predentin; and the outer darker staining region representing the mature calcified dentin. The discrete characteristics of interglobular dentin are believed to be the result of a failure of the globules to coalesce. The experimental animals present an increased number of these acidophilic poorly calcified areas (Fig. 2).

Metachromasia.—The incisal dentin from control animals was not metachromatic when stained with toluidin blue, rather appearing to give an orthochromatic response to this dye (Fig. 3). In contrast, the dentin of the incisors of the experimental animals was highly metachromatic when similarly treated (Fig. 4). The metachromatic areas were not uniform, appearing to be more intense in regions of accentuated disturbed calcification such as in the interglobular spaces. These metachromatic properties could be produced in the incisal dentin of control animals after subjecting the blocks of tissue to peptic digestion (Fig. 5).

Periodic Acid-Schiff Reaction.—The color of the normal dentin after being stained by the periodic acid-Schiff method is light pink, this staining reaction being homogenous throughout the dentin (Fig. 6). The ground substance of the dentin from the deficient animals responds in a different manner. Fig. 7 is a section of the incisal dentin from an animal that was on the tryptophan-deficient diet for 7 weeks. A narrow outer area (*Dp.*) is observed which stains light pink and represents the dentin deposited during the predeficient dietary period. The wide inner layer, the dentin formed during the deficient period,

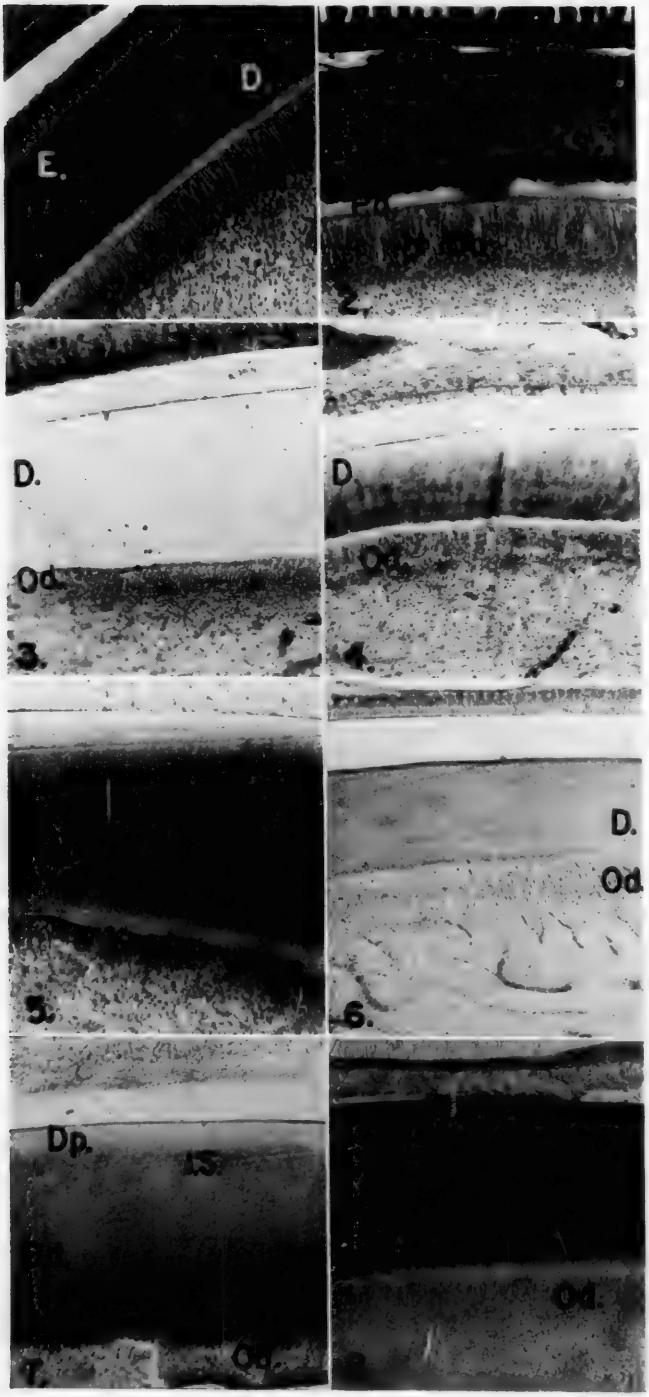


Fig. 1—*(For legend, see opposite page.)*

stains a deep red. However, this staining reaction is not uniform in intensity throughout. The more intensely stained regions are the poorly calcified interglobular spaces. The removal or suppression of the collagenous fibers after peptic action produces a histochemical picture analogous to that found in the deficient animals (Fig. 8).

Alkaline Phosphatase Reaction.—The odontoblastic layer and the dentin of the control incisors exhibited an intense response to the Gomori's technic (Fig. 9). To distinguish between preformed and enzymatically deposited phosphate, alternate sections from the control animals were treated by the same procedure but the enzymatic substrate was omitted. The negative results that were obtained under such conditions are illustrated in Fig. 10. The dentin as well as the odontoblastic layer show a negative reaction. The absence of tryptophan in the diet appears to alter the reaction of the odontoblasts and dentin to the Gomori's technic (Fig. 11). It is interesting to note that areas of negative periodic acid-Schiff reaction such as the interglobular spaces similarily are negative to the Gomori's method. The remaining dentin matrix and odontoblasts stain fainter than do the control dentin and cells. It is also interesting to note that in this case the use of peptic digestion of tissue blocks from control animals resulted in an increased response in the dentin rather than a decrease. This may be accounted for by the possibility that pepsin digest away interfering protein from the specific staining group and thus increase the number of available active centers.

Electron Microscopy Studies.—Photomicrographs of dentin from the control animals reveal the dentinal tubule containing the protoplasmic process of the odontoblast surrounded by a matrix which consists of vacuoles, ground substance, and collagenous fibrils of 640 A. spacings. The vacuoles represent the regions in which the minerals were present before the process of decalcification. The collagenous fibrils do not show clearly, probably due to the masking by mucoproteins (Fig. 13). On the other hand when one studies photomicrographs of dentin from deficient animals, the collagenous fibrils are

---

Key to abbreviations in Figs. 1–14.

D., dentin	*I.S.*, interglobular space
Dd., deficient dentin	*E.*, enamel
Dp., predeficient dentin	*Pd.*, predentin
D.T., dentinal tubule	*Od.*, odontoblastic layer

Fig. 1.—Hematoxylin and triosin stained section of an upper incisor from control animal. Note homogeneity of dentin. Orig. mag. ×300.

Fig. 2.—Hematoxylin and triosin stained section of an upper incisor from an animal that was on tryptophan-deficient diet. Note increased number of interglobular areas in dentin. Orig. mag. ×300.

Fig. 3.—Section of incisal dentin and pulp from control animal stained by 1 per cent toluidin blue. Dentin failed to show metachromasia. Orig. mag. ×300.

Fig. 4.—Section of incisal dentin and pulp from experimental animal stained by 1 per cent toluidin blue. Dentin now exhibits metachromasia with the interglobular areas showing an intense reaction. Orig. mag. ×300.

Fig. 5.—Section of incisal dentin and pulp from control animal that was pretreated with 1 per cent pepsin. Dentin now shows metachromasia. Compare with Fig. 3. Orig. mag. ×300.

Fig. 6.—Section of incisal dentin from control animal that was stained by periodic acid-Schiff method. Dentin, as well as the odontoblastic layer, stain light pink. Note homogenous appearance of dentin. Orig. mag. ×300.

Fig. 7.—Section of incisal dentin from experimental animal that was stained by periodic acid-Schiff method. Observe 2 layers in dentin. Outer region (*Dp.*) represents dentin deposited before deficiency while inner area (*Dd.*) is deficient dentin. Orig. mag. ×300.

Fig. 8.—Section of incisal dentin from control animal that was pretreated with pepsin. Note intense staining reaction after being stained by periodic acid-Schiff method. Orig. mag. ×300.

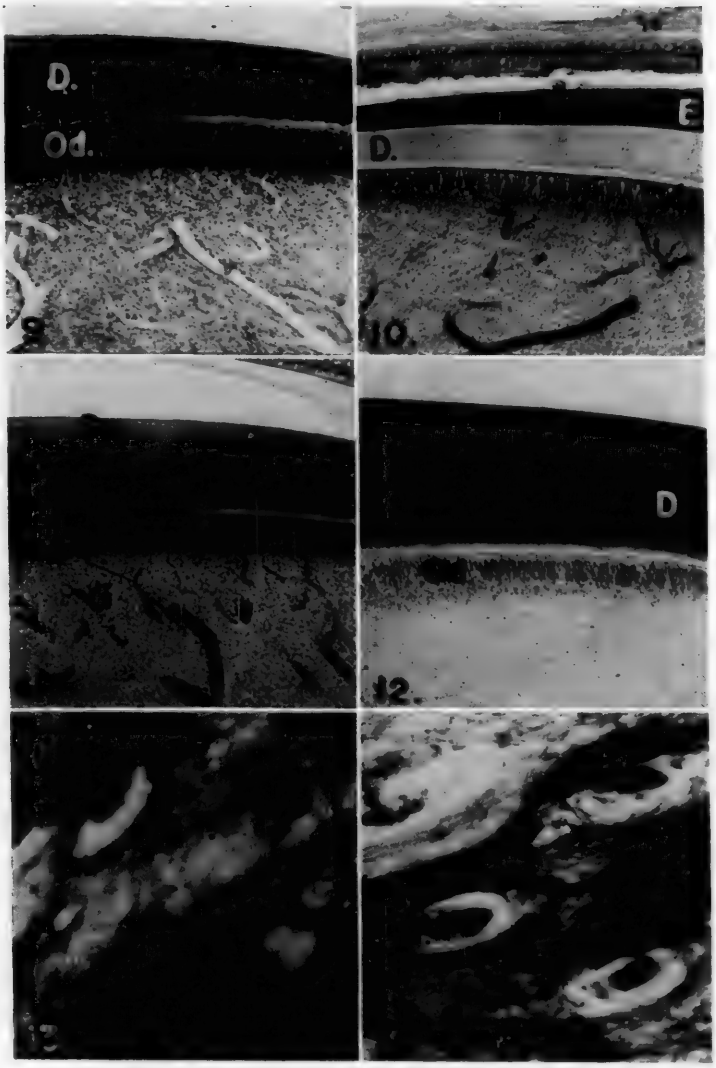


Fig. 9.—Control section of incisal dentin that was stained by Gomori's alkaline phosphatase method. Note intense positive reaction in dentin and odontoblastic layer. Orig. mag. ×300.

Fig. 10.—Control section of incisal dentin that was incubated without glycerophosphate. Compare with Fig. 9. Note absence of response in dentin and odontoblastic layers. Orig. mag. ×300.

Fig. 11.—Section of incisal dentin from experimental animal that was stained by Gomori's alkaline phosphatase method. Note decrease in staining intensity in both the dentin and pulp. Orig. mag. ×300.

Fig. 12.—A control section that was pretreated with pepsin. Note that after being exposed to Gomori's technic dentin stains deep black. Orig. mag. ×300.

Fig. 13.—Photomicrographs of dentin from control animal. Note dentinal tubule surrounded by organic matrix. Collagen fibrils of 640 A. spacings are not clearly visible probably due to the masking by the highly polymerized protein mucopolysaccharide complex. Orig. mag. ×15,000.

Fig. 14.—Photomicrograph of dentin from tryptophan-deficient animal. Note orientation of collagenous fibrils of 640 A. spacings in matrix. Orig. mag. ×15,000.

clearly visible, and one may observe their orientation in the matrix. It appears that these fibrils encircle the dentinal tubule thus participating in the circumscription of the odontoblastic process (Fig. 14).

DISCUSSION

The process of dentinogenesis is initiated by the appearance of an uncalcified matrix composed primarily of precollagenous fibrils and a protein mucopolysaccharide ground substance. These precollagenous fibrils are soon transformed into collagenous fibers as one of a series of changes which appear to produce a receptive matrix for the mineralization of the dentin. This mineralization is not uniform but occurs in isolated rounded clusters of calcium apatite crystals known as calcospherites. These calcospherites eventually tend to aggregate and coalesce to form larger calcoglobules which finally fuse to form a homogenous incremental layer of dentin. The failure of fusion of these calcoglobules results in the production of acidophilic interglobular spaces. Any disturbance in the formation of the dentin by a deficiency of either inorganic or organic elements will result in an increased number of these areas. Thus an increase in these interglobular areas was noted in all the animals in these experiments regardless of age or duration of the deficiency. Inasmuch as the mineral intake was unaltered one may assume that the disturbance in calcification may be related to degenerative changes in the organic matrix.

The property of metachromasia and the reaction to periodic acid-Schiff stain allows one to identify and observe the state of the ground substance of calcified tissue. Engel[9] and Burstone,[7] using the periodic acid-Schiff reaction, demonstrated that mature dentin stains a light pink indicating that its ground substance is in a high state of polymerization. On the other hand they concluded that the predentin was less polymerized as evidenced by a deeper staining response. Burstone,[7] in addition, studied pathological dentin, secondary dentin, and interglobular dentin and came to the conclusion that in these abnormal conditions the ground substance was in a depolymerized state. Bevelander and Johnson[6] using both toluidin blue and periodic acid-Schiff reaction on developing dentin confirmed both Engel's and Burstone's results. Levine and Schubert[12] demonstrated chemically that an increase in calcium ions in combination with chondroitin sulfate reduces or abolishes metachromasia. This finding would explain the negative reactions that the above workers obtained when they studied mature dentin. The results of the present study agree with the findings of the above investigators, that is, when mature dentin from control animals was exposed to either toluidin blue or periodic acid-Schiff reaction, the staining results indicated a highly polymerized state. On the other hand the deficient dentin stained highly metachromatic after toluidin blue and deep red with periodic acid-Schiff reaction. This staining

behavior would imply that the absence of tryptophan has so altered the calcifiability of the ground substance as to inhibit its polymerization. The suggestions of Rubin and Howard,[13] Heller-Steinberg,[11] Cobb,[8] and Bevelander and Johnson[5] that the state of the ground substance is related to the calcifiability of bone and dentin more or less confirms this implication.

The role of alkaline phosphatase in the process of calcification is one which to this day is not clearly defined. However, the enzyme has been localized in both the odontoblastic layer and dentin (Bevelander and Johnson,[4] and Harris[10]). In the present study, differences were noted between the dentin of the control animals and that of the other experimental animals in their reaction to the Gomori's technic. It is questionable to what extent these differences are related to the presence of alkaline phosphatase inasmuch as the specimens were fixed in alcoholic Bouin's solution and decalcified in nitric acid; procedures which should inactivate the enzyme. On the other hand, the dentin from either control sections that were incubated without glycerophosphate or deficient animals gave a negative or faint response, respectively, whereas the control sections stained in a positive manner, following treatment with the Gomori's method.

The enzymatic hydrolysis of normal dentin was undertaken for two principal reasons. It is well known that collagen makes up a large quantity of the dentin, and this compactness of the collagenous elements may mask the reactivity of the groups of the ground substance to the stains used. The second intention was to attempt to duplicate artificially the defective protein synthesis noted in the experimental animals by enzymatic digestion of the protein component in the dentin of the control animals. The findings obtained showed that this peptic digestion resulted either in the removal of the collagenous elements or the suppression of their staining reaction. At the same time there was an enhanced staining intensity of the ground substance to both toluidin blue and periodic acid-Schiff reaction. These histochemical changes were similar to those found in the experimental animals.

Electron microscopy studies of the fibrillar component of the dentin have not been particularly successful in demonstrating their orientation in the organic matrix. The main difficulty seems to be the highly polymerized ground substance that appears to mask these fibrils. However, photomicrographs of deficient dentin reveal clearly the presence of these collagen fibrils of 640 A. spacings. The failure of the protein mucopolysaccharide synthesis probably prevents the progressive polymerization normally occurring during calcification and consequently these fibrils are unmasked. Thus at this time one may observe the patterns of these fibrils in the matrix. These electron microscopy findings appear to substantiate the histochemical observations.

SUMMARY

In the present investigation histological sections of the incisors from control and tryptophan-deficient rats were treated by various histochemical methods and electron microscopy in an effort to detect changes in the dentin

that would explain the failure of this tissue to calcify in the absence of tryptophan. The results of the staining reaction, employing the periodic acid-Schiff method, toluidin blue, and Gomori's method, indicated that the ground substance was in a depolymerized state due to the absence of tryptophan, thus preventing normal calcification of the dentin to take place.

Electron microscopy studies of the dentin from the control and from tryptophan-deficient animals showed morphological differences. In the deficient animals the orientation of the collagen fibrils of 640 A. spacings in the dentin were visible whereas in the normal dentin these fibrils were masked by the unaltered ground substance.

Pepsin hydrolysis of control incisal sections resulted in the enhancing of the staining intensity of the ground substance to both toluidin blue and periodic acid-Schiff reaction. These histochemical changes were similar to those found in the experimental animals.

The authors wish to thank Dr. R. Baker, Department of Experimental Medicine for his aid in the electron microscopy studies.

REFERENCES

1. Bavetta, L. A., and Bernick, S.: The Effect of Tryptophan Deficiency Upon the Jaws of Rats, *J. D. Res.* **33**: 309, 1954.
2. Bernick, S. Baker, R., Rutherford, R. L., and Warren, O.: Electron Microscopy of Enamel and Dentin, *J. A. D. A.* **45**: 689, 1952.
3. Bernick, S.: Innervation of the Teeth and Jaws of the Rat, *Anat. Rec.* **125**: 185, 1956.
4. Bevelander, G., and Johnson, P. L.: A Histochemical Localization of Alkaline Phosphatase in the Developing Tooth, *J. Cell. & Comp. Physiol.* **26**: 25, 1949.
5. *Idem:* A Histochemical Study of the Development of Bone, *Anat. Rec.* **108**: 1, 1950.
6. *Idem:* The Localization of Polysaccharides in Developing Teeth, *J. D. Res.* **34**: 123, 1955.
7. Burstone, M. S.: The Ground Substance of Abnormal Dentin, Secondary Dentin, and Pulp Calcification, *J. D. Res.* **32**: 269, 1953.
8. Cobb, J.: The Morphological Distribution of Glycogen and Glycoproteins in Cells and Extracellular Material in Bone, Thesis, University of Illinois, 1949.
9. Engel, M.: Glycogen and Carbohydrate Protein Complex in Developing Teeth of the Rat, *J. D. Res.* **27**: 681, 1948.
10. Harris, E.: Sites of Alkaline Phosphatase in Dentinogenesis, *Anat. Rec.* **107**: 105, 1950.
11. Heller-Steinberg, M.: Ground Substance, Bone, Salts, and Cellular Activity in Bone Formation and Destruction, *Am. J. Anat.* **89**: 347, 1951.
12. Levine, A., and Schubert, M.: Metachromasia of Thiazine Dyes Produced by Chondroitin Sulfate, *J. Am. Chem. Soc.* **74**: 91, 1952.
13. Rubin, P. S., and Howard, J. E.: Histochemical Studies on the Role of Acid Mucopolysaccharides in Calcifiability and Calcification, Trans. Josiah Macy Conference on Metabolic Interrelations, 1950.
14. Scott, D. B.: The Electron Microscopy of Enamel and Dentin, *Ann. New York Acad. Sc.* **60**: 575, 1955.

THE RELATION OF OXYGEN PRESSURE TO ENDOGENOUS OXYGEN CONSUMPTION IN BOVINE DENTAL PULP

ALTON K. FISHER, ERWIN R. SCHUMACHER, NORMAN P. ROBINSON, DELMAR D. ALBERS, AND GARY P. SHARBONDY

Department of Oral Pathology, College of Dentistry, State University of Iowa, Iowa City, Iowa

THE RATE of respiration in a tissue may conveniently be measured by observing the amount of oxygen consumed by a known mass of that tissue during a definite period of time. This rate is usually stated as the Oxygen Quotient (QO_2) which conventionally expresses the number of microliters of oxygen utilized by a milligram of tissue in one hour. The Warburg technic commonly employed in studies of tissue respiration entails the use of thin tissue slices, minces or homogenates, suspended in appropriate solutions and contained within flasks connected to manometers designed to reflect the gas pressure or gas volume changes within the system. The flask-manometer system is maintained at constant temperature during use by a water bath, and is also subjected to constant shaking to ensure adequate diffusion of respired gases through the suspending fluids.

It is often assumed that the gas phase above the suspended tissue slices need contain an amount of oxygen only modestly in excess of the requirements of the tissue during the period of observation. It is believed as a general physiologic principle that, "Increase in the oxygen supply to the tissue . . . does not increase the oxygen usage."[1] Dixon, in referring to manometic studies stated that, ". . . in general the rate of cell respiration remains independent of oxygen pressure until the latter is reduced to a small fraction of an atmosphere. . . ."[2] Air has frequently been employed as the gas phase in respiration studies. Where contaminating gases in the air might possibly interfere with tissue reactions, a mixture of pure nitrogen and oxygen in proportions approximating that of air has been substituted. In other instances, a gas phase containing mainly oxygen has been chosen. There are reports, however, "that some types of respiration are affected by the pressure of oxygen, *per se*,"[3] although data on this matter are not numerous.[4]

Flieder and Fisher[5] reported the QO_2 for normal bovine dental pulp to be 0.56 (S.D. ±0.13). The gas phase used in that study was oxygen, and the reaction flasks were of 5 ml. capacity. The present study was undertaken to determine whether or not the use of air as the gas phase would influence the observed QO_2 of the same tissue.

This investigation was supported in part by Research Grant No. D-126 from the United States Public Health Service.

Received for publication Sept. 22, 1955.

EXPERIMENTAL METHOD AND RESULTS

The experimental conditions in this study were identical to those employed by Flieder and Fisher[5] except that air was employed as the gas phase in 2 sets of observations using reaction flasks of 5 ml. and 15 ml. capacity, respectively, while oxygen was the gas phase in a third set of observations using 15 ml. flasks. The method of computing the flask constants in the present study utilized gas volume determinations as described by Lazarow,[6] thus eliminating an element of assumption relative to specimen volume, and consequently of gas volume (Vg).

A summary of the results of this and the previous study of the QO_2 of normal bovine dental pulp is presented in Table I. When the determinations in Series II, using 5 ml. reaction flasks with air for the gas phase, indicated a mean QO_2 of 0.33 it was thought that the flasks might be overloaded with tissue in respect to the total volume of available oxygen. Since the total volume of oxygen consumed in each determination was only a small fraction of the available supply, however, this explanation did not seem reasonable.

TABLE I

SERIES NO.	NUMBER OF DETERMINATIONS	GAS PHASE	MEAN VG (ML.)	MEAN VO_2	MEAN pO_2	MEAN QO_2	S.D.	RANGE
I Flieder and Fisher[5]	54	O_2	4.6	4.6	745	0.56	0.13	0.26-0.90
II	19	Air	4.6	0.9	149	0.33	0.08	0.11-0.67
III	12	Air	13.6	2.7	149	0.47	0.22	0.12-0.80
IV	24	O_2	13.6	13.6	745	0.54	0.15	0.30-0.93

The determinations in Series III, using 15 ml. flasks with air as the gas phase, suggested a higher QO_2 than was evident in Series II but the mean value was still below that indicated for Series I. In Series IV, the QO_2 values correspond closely to those obtained in Series I even though a higher oxygen volume (VO_2) was used in the last series.

Inspection of Table I suggests that oxygen pressure (pO_2) was the factor among those considered in these measurements that modified the observed QO_2 in normal bovine dental pulp. Reconsideration of the data on a gas phase basis is summarized in Table II.

TABLE II

GAS PHASE	NUMBER OF DETERMINATIONS	MEAN pO_2	MEAN QO_2	S.D.	RANGE
O_2	78	745	0.55	0.14	0.26-0.95
Air	31	149	0.37	0.20	0.11-0.80

DISCUSSION

The literature dealing with manometric methods contains abundant consideration of the problems relating to the diffusion of respired gases through

tissue specimens and suspending fluids. The rate of diffusion of oxygen is partially dependent on its pressure. On a simple theoretic basis it might be assumed that the partial pressure of oxygen in an ample volume of air is adequate for the respiratory requirements of dental pulp in view of its low QO_2. Evidently this is not true.

Warburg's formula[2] relative to the maximum permissible thickness of tissue slices may be expressed as follows:

$$d' = \sqrt{8cO\frac{D}{A}}$$

In this equation d′ is the thickness of the tissue slice in centimeters, cO is the oxygen concentration in atmospheres, D is the diffusion constant of oxygen in tissue and A is the respiratory rate expressed as milliliter per minute per cubic centimeter of tissue. If a pO_2 of 1.0 and a QO_2 of 0.56 are used as a basis of calculation it will be found that the maximum permissible thickness of bovine pulp tissue slices is 3.5 mm. If, instead, the pO_2 employed is 0.2 and the QO_2 is 0.33, the maximum slice thickness is calculated to be 2.0 mm. But under the conditions of this investigation, the thickness of tissue slices was always below 1 mm. and usually about 0.5 mm.

It seems evident, therefore, that the varied respiratory activity of bovine dental pulp under varied conditions of oxygen pressure is not attributable to diffusion factors originating in unduly thick tissue slices. It appears that the higher mean QO_2 obtained with a pO_2 approximating 1 atmosphere reflect physiologic peculiarities of that tissue which, at present, lack adequate explanation.

CONCLUSION

This study has indicated that maximum mean QO_2 values for normal bovine dental pulp suspended in Krebs-Ringer-phosphate solution are not obtainable with a gas phase of air. It has succeeded in closely approximating the results of earlier QO_2 determinations of the same tissue with a gas phase of oxygen. The original and present QO_2 values are 0.56 (S.D. ±0.13) and 0.54 (S.D. ±0.15), respectively.

REFERENCES

1. Best, C. H., and Taylor, N. B. The Physiologic Basis of Medical Practice, ed. 4, Baltimore, 1945, The Williams & Wilkins Company, p. 322.
2. Dixon, M.: Manometric Methods, ed. 3, Cambridge, 1951, Cambridge University Press, p. 57.
3. Umbreit, W. W., Burris, R. H., and Stauffer, J. F.: Manometric Techniques and Tissue Metabolism, ed. 2, Minneapolis, 1949, Burgess Publishing Co., pp. 10-11.
4. Höber, R.: Physical Chemistry of Cells and Tissues, Philadelphia, 1945, The Blakiston Company, p. 383.
5. Flieder, D. E., and Fisher, A. K.: The Rate of Endogenous Oxygen Consumption in Bovine Dental Pulp, *J. D. Res.* **34**: 921, 1955.
6. Lazarow, A.: A Calibrator for Determining the Volume of the Warburg Vessel Plus Manometer, *J. Lab. and Clin. Med.* **38**: 767-772, 1951.

EFFECT OF 1,2,5,6-DIBENZANTHRACENE ON TRANSPLANTED TOOTH GERMS

HAROLD S. FLEMING, D.M.D., M.Sc., D.Sc.

From the Department of Pathology, Yale University School of Medicine, New Haven, Conn.

INTRODUCTION

NEOPLASMS have been induced by applying polycyclic hydrocarbons to the skins of several species of experimental animals and birds.[2, 19] These compounds have been injected subcutaneously into animals with the aim of determining their persistence at the injection site as well as their ultimate distribution and excretion.[7, 14, 15, 18] While carcinogenic polycyclic hydrocarbons have been extensively studied, under varying conditions, their metabolic pathways and how they exert specific effects remain obscure.[3-7, 9, 15-17, 23, 26] It is generally agreed, however, that they interfere with some phase of sulfur metaboloism.[9, 14, 20, 23, 27] Transplanted embryonic tissues have been treated with crystals or small particles of these carcinogenic hydrocarbons and neoplasms that met some or all of the criteria for malignancy have been produced.[11-13, 21, 22]

In previous experiments, 20-methlycholanthrene appeared to have a greater effect on the epithelial than on the mesenchymal tissues of tooth germ transplants.[11, 12] Some investigators have reported that 20-methylcholanthrene takes 2 to 3 months to induce carcinogenesis, while the period required for 1,2,5,6-dibenzanthracene to produce similar effects takes at least 6 to 7 months.[14] It has also been demonstrated that embryonic tissues do not respond similarly to these carcinogenic hydrocarbons.[21, 22]

The purpose of this communication is to report the effect of 1,2,5,6-dibenzanthracene on transplanted tooth germs and to compare it with the effect of 20-methylcholanthrene. Certain benign as well as malignant neoplasms are known to arise from the embryonal tissues of the teeth.[24, 25] Therefore these studies may be considered additional attempts to induce formation of benign or malignant neoplasms by treating transplanted embryonic tooth tissues with polycyclic hydrocarbons of known carcinogenic proclivities.

MATERIALS AND METHODS

Tooth germs of a known age were obtained from guinea pigs, rabbits, and dba mice embryos. Technics of transplantation as well as the use of carcinogenic hydrocarbons with embryonic tissues have been previously reported.[10-13]

This study was made possible through a grant from the Department of Health, Education, and Welfare, United States Public Health Service. A preliminary report was given on this subject at the 32nd General Meeting of the I. A. D. R. at French Lick Springs, Ind., March 19 to 21, 1954 (*J. D. Res.* **33**: 657, 1954).

Received for publication Aug. 8, 1955.

Homo- and heterotransplants were made to the eyes and brains of guinea pigs, to the axillae and brains of dba mice and to the brains of rabbits. Dibenzanthracene crystals were also mixed with sterile agar and placed at the transplantation sites.

Tooth germ transplants treated with this polycyclic hydrocarbon were allowed to remain in place from 4 to 407 days. With each series a number of untreated transplants were likewise made to serve as controls. Animals were sacrificed intermittently and recovered tissues were fixed, decalcified when necessary, sectioned, and stained for histologic study. Hematoxylin and eosin were generally used for staining; however, Masson's and the Barrnett-Seligman stains were also employed.[12] Autopsies were routinely made on animals that were periodically sacrificed as well as on those that died or appeared moribund and had to be killed. In some cases recovered tissues were retransplanted and when possible carried serially.

RESULTS

1,2,5,6-dibenzanthracene appeared to have an early toxic effect on some of the hosts bearing brain transplants. When brain transplants were made, several animals died within 24 to 48 hours following the operation. This was not experienced with normal brain controls, nor when methylcholanthrene was similarly used. Gross and histologic examination of these animals' brains showed hemorrhagic areas at the transplantation sites.

In the livers and kidneys of hosts bearing dibenzanthracene-treated transplants there were occasional abscess formations. It is not certain, however, whether these changes could be attributed to the presence of this compound or to its metabolic by-products. In some instances atypical cells were found in both livers and lungs. Over longer periods of these experiments, kidneys frequently showed degenerative changes often with metastatic calcification. Lungs were often consolidated and showed hyperemia of vessels.

Treated transplants recovered at various periods during these experiments did not attain the same gross size as those made with methylcholanthrene and recovered after similar periods. From 4 to 15 days following transplantation there was considerable mitotic activity in the pulpal areas of transfers (Fig. 1). This was not true for later periods. In these regions there were also a number of free red blood cells (Fig. 2). The initial response of the host was inflammation, aggregations of polymorphonuclear leukocytes and monocytes appearing adjacent to the transplants soon after transplantation. In later periods small foci of lymphocytes were found near the transfers. Frequently, by about 80 days following transplantation, adjacent blood vessels were hyperemic and some were plugged with a serous coagulum. About this time in other nearby areas, masses of free red blood cells were also found.

Some of these transplants showed evidences of calcification as early as 18 to 20 days following transplantation. This was also observed with normal control transplants for similar periods. Cells of the outer enamel epithelium, in treated transplants, rapidly dedifferentiated in the absence of the formation of calcified tooth structures. In places, layers of epithelial cells were found

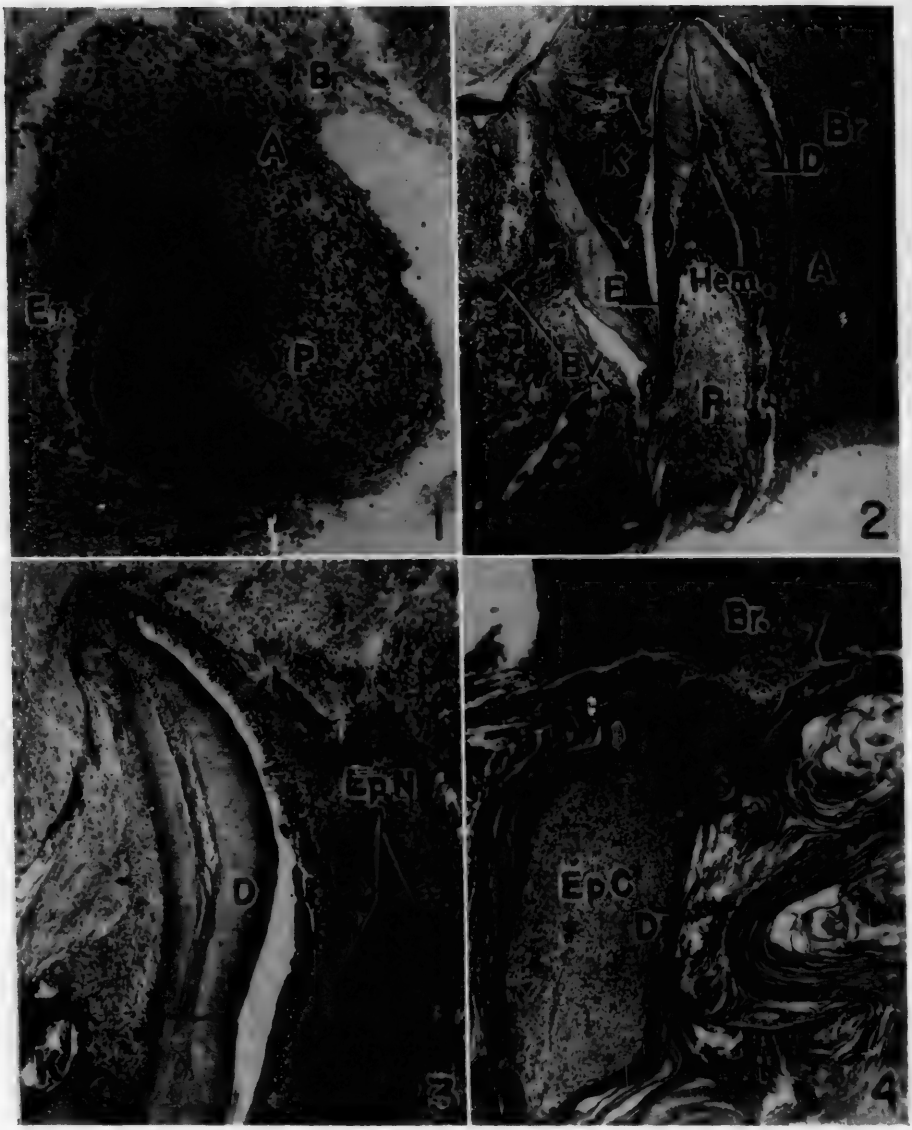
sometimes 6 to 8 or more cells deep (Fig. 3). At this period there was a proliferation of some cells away from the main body of the transfers. Nests of epithelial cells were also observed and in some instances cystic areas had begun to form (Fig. 4). Polymorphonuclear leukocytes were now less frequently seen, but small foci of lymphocytes were found. In some cases there was a degeneration of the transplants.

By 30 days keratin, which stained lightly with eosin, was generally observed (Fig. 5) and cystic areas now appeared larger. Some of these cystic areas had coalesced with each other and in this manner had formed polycystic regions. By 70 to 80 days keratin stained much heavier and was often observed arising from outer borders of multilayered epithelial cells, many of which lay adjacent to formations of tooth structures (Figs. 6 and 7). Frequently these tooth structures consisted of thin strips of dentin. Enamel had failed to form (Fig. 6). In these instances ameloblasts appeared to have undergone degenerative changes, appearing shrunken and stained heavily with hematoxylin. More frequently from 80 days upward, nests of epithelial cells in palisaded arrangement broke down in the centers and new cells seemed to be arising from the peripheral borders. For periods of 120 days and upward keratin, in some places, appeared amorphous and, adjacent to it, epithelial cells seemed to be forming new layers (Fig. 8). This keratin did not appear to stretch or unfold to the great length (Fig. 9) it did in the presence of methylcholanthrene. For these longer periods, calcification was poor and dentin was often associated with cartilagenous structures.

In several instances tissues recovered after more than 150 days was retransplanted. These transplants were originally homotransfers to the brains of guinea pigs, and when this tissue was retransplanted it was transferred to the eyes of animals of the same species. Some of these transfers have been carried serially for a period of over 18 months and are still growing. The original tissues retransplanted consisted of cells of an epithelial nature, exhibiting variations in size. Numerous cells in various stages of mitosis were observed in these sections and the nuclei of the majority of the other cells were hyperchromatic. Some of these cells arranged in cords or chains were invading adjacent brain tissue of the host (Figs. 11 and 12).

There was resorption of dibenzanthracene-treated brain homotransplants of rabbit tooth germs. These results were obtained for varying periods up to a year. No traces of the transplants were found in any instance and only slight hemorrhagic regions marked the transplantation sites. The transfer sites of dibenzanthracene and sterile agar were likewise hard to locate and often only a slight increase in vascularity was all that indicated where the transplant had been made.

For the periods of these studies with dibenzanthracene and tooth germ transplants the ameloblasts, or cells of the inner enamel epithelium, degenerated when the enamel matrix was not laid down and cells of the stratum intermedium dissociated themselves and appeared to migrate into adjacent



Figs. 1 to 12.—Key to symbols: *A* = Ameloblasts, *Br.* = Brain, *BV* = Blood vessel, *Ch.S* = Cholesterol slits, *D* = Dentin, *E* = Enamel, *Ep.C* = Epithelial cells, *Ep.N* = Epithelial nests, *Er.* = Erythrocytes, *F* = Fat, *H* = Hemorrhagic area, *K* = Keratin, *Mi.* = Mitosis, *O* = Odontoblasts, *OD* = Osteodentin, and *P* = Pulp.

Fig. 1.—Dibenzanthracene-treated transplant recovered after 4 days in the brain of a female guinea pig. Original tissue obtained from a 25-day-old guinea pig embryo. (Original magnification ×240, reduced about 2/7.)

Fig. 2.—Hemorrhagic areas and plugging of blood vessels in dibenzanthracene-treated transplant recovered after 46 days in the brain of a male guinea pig. Original tissue obtained from a 35-day-old guinea pig embryo. (Original magnification ×75, reduced about 2/7.)

Fig. 3.—Nests of epithelial cells associated with tooth structure in dibenzanthracene-treated transplant after 28 days in the brain of a male guinea pig. (Original magnification ×100, reduced about 2/7.)

Fig. 4.—Cystic areas and poor dentin formation in a dibenzanthracene-treated transplant recovered after 50 days in the brain of a female guinea pig. A 30-day-old guinea pig embryo supplied the original tissue. (Original magnification ×40, reduced about 2/7.)

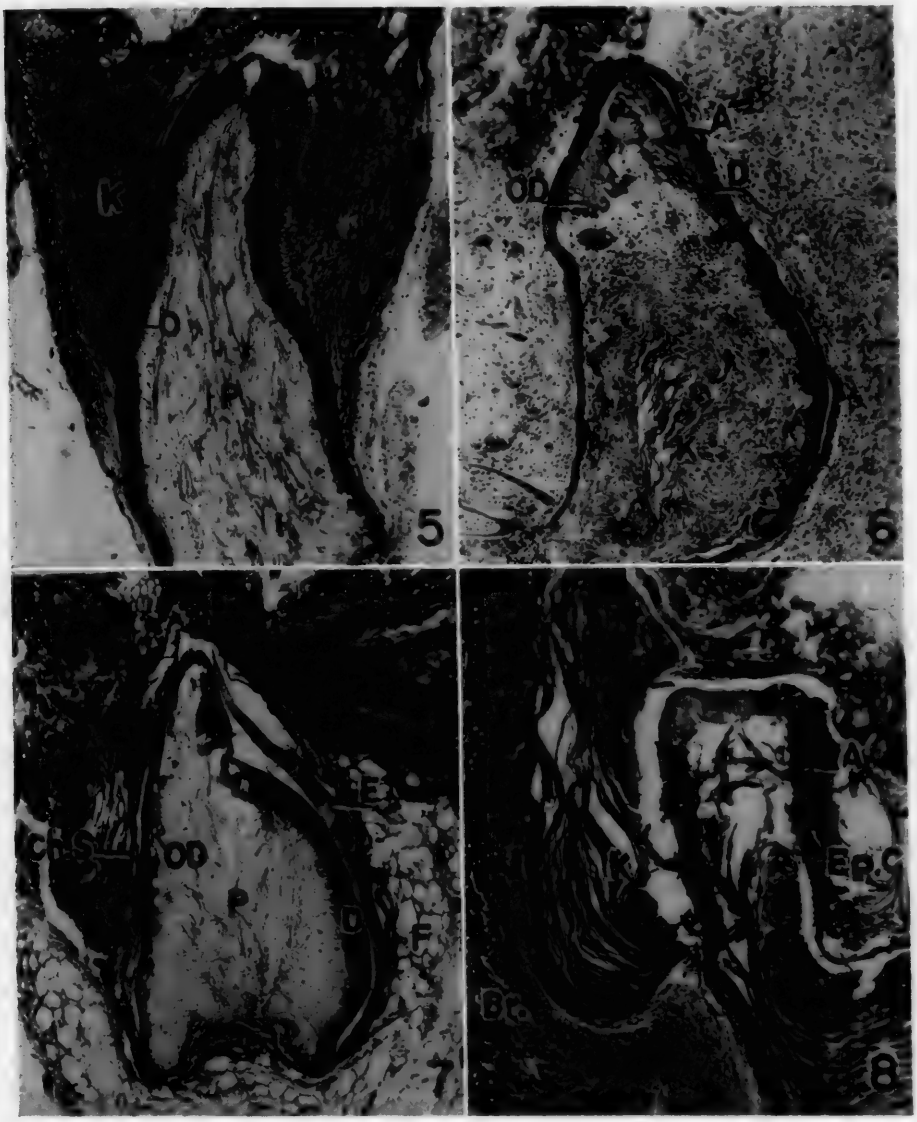


Fig. 5.—Retarded maturation and development in a dibenzanthracene-treated transplant after 67 days in the axilla of a dba male mouse. Original tissue was obtained from a 22-day-old rabbit embryo. (Original magnification ×130, reduced about ⅔.)

Fig. 6.—Osteodentin and degeneration of ameloblasts in a recovered dibenzanthracene-treated transplant after 55 days in the brain of a male guinea pig. Original tissue was obtained from a 30-day-old guinea pig embryo. (Original magnification ×130, reduced about ⅔.)

Fig. 7.—Cholesterol slits in a dibenzanthracene-treated transplant after 67 days in the axilla of a dba male mouse. Original tissue was obtained from a 15-day-old dba embryo. (Original magnification ×140, reduced ⅔.)

Fig. 8.—Disorganized epithelial cells adjacent to tooth structure in a dibenzanthracene-treated transplant after 115 days in the brain of a male guinea pig. Original tissue was from a 28-day-old guinea pig embryo. (Original magnification ×75, reduced ⅔.)

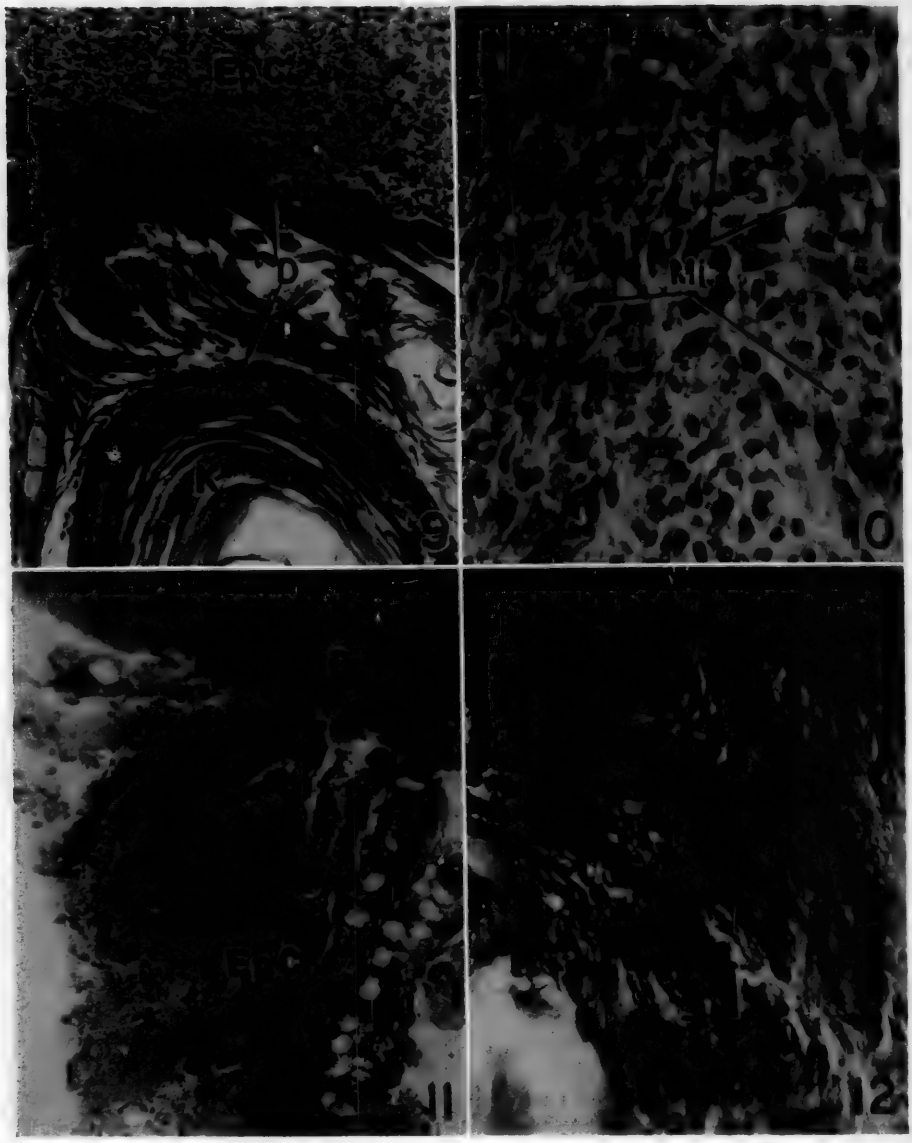


Fig. 9.—Epithelial cells with marked cellular activity adjacent to keratin and poor dentin formations in a dibenzanthracene-treated transplant after 66 days in the eye of a male guinea pig. Original tissue was obtained from a 30-day-old guinea pig embryo. (Original magnification ×275, reduced about 2/7.)

Fig. 10.—Area of epithelioid cells from same section as Fig. 9, showing pleomorphism and marked cellular activity with numerous mitotic figures. (Original magnification ×740, reduced about 2/7.)

Fig. 11.—Recovered brain transplant of dibenzanthracene-treated tissue after 98 days in the brain of a male guinea pig. There is no localized zone of growth and cells are breaking through into brain tissue in adjacent areas. (Original magnification ×325, reduced about 2/7.)

Fig. 12.—Dibenzanthracene-treated transplant after 142 days in the brain of a male guinea pig. Original tissue was from a 30-day-old guinea pig embryo. This tissue was transplanted and carried serially for more than a year. (Original magnification ×300, reduced about 2/7.)

tissues. These latter cells did not participate in the production of keratin. There did not appear to be any significant difference in the results between homotransplants and heterotransplants in these experiments except as indicated with rabbit brain homotransplants.

DISCUSSION

Epithelial tissues of transplanted tooth germs, particularly those of the outer enamel epithelium, were affected most by 1,2,5,6-dibenzanthracene or its metabolic by-products. Under conditions of these experiments, developing independently of tooth structures, keratin and cystic areas were frequently found. In addition, other tissues that had the histologic and biologic requirements for malignancy were occasionally recovered. Keratin as found in the present instance, except for its shorter length, was similar to that found when methylcholanthrene was used. Staining for protein-bound SH groups was also histologically positive in these experiments. The amounts of keratin, however, were not as abundant as when methylcholanthrene was used, and in addition tumors produced by this latter substance were more discrete and larger. In the experiments with these polycyclic hydrocarbons it was apparent that keratin, from its relation to the transplants, usually arose from cells of the outer enamel epithelium. It is also possible that some could have come from the ameloblasts.[11, 12]

Of the polycyclic hydrocarbons, 20-methylcholanthrene is considered by some investigators to have a higher carcinogenic potency than 1,2,5,6-dibenzanthracene. It has also been estimated that the induction period for this latter substance is much longer.[14] The findings for dibenzanthracene, except as indicated above, did not differ from those previously observed with methylcholanthrene.[11, 12] This is in accord with Bryan and Shimkin[8] who compared the effects of small doses of dibenzanthracene and methylcholanthrene.

Lipoids of the cells represent pathways through which hydrocarbons are able to penetrate their most intimate structure; and thiol groups in proteins are in some way affected by these carcinogenic hydrocarbons.[20] A carcinogen-enzyme complex results when carcinogenic hydrocarbons become fixed to accessible SH groups.[1, 23] Thus specific cellular enzymes, which in their activity control growth and development, are altered and ultimately a new type of protein with autosynthetic properties is formed within the cell.[9] Under conditions of the present experiments there was an alteration in the behavior of the cells of the outer enamel epithelium which was manifested by the continued formation of keratin. Further evidence in the alteration of growth and behavior was depicted by those epithelial cells which, failing to form keratin, attained certain malignant characteristics. These cells presumably originating from dedifferentiated outer enamel epithelium seemed to have the potentiality to acquire aggressive tendencies when interfered with during growth and development. This offers a possible explanation relative to the frequent appearance of cysts associated with developing teeth and also to the much less frequent central carcinomas of the jaws. This dissociation of

outer enamel epithelium from the rest of the tooth germ may be easily accomplished. Following this, nests or cords of cells are formed which in the presence of certain compounds or under the influence of particular stimuli may result in uncontrolled growth.

CONCLUSION

1. Under conditions of the present experiments, 1,2,5,6-dibenzanthracene seemed to have its greatest effect on the epithelial tissues of tooth germ transplants.

2. Keratin formations were smaller and less well defined than with 20-methylcholanthrene.

3. Recovered transplants sometimes met histologic and biologic requirements for malignancy.

4. Growth and development of tooth germ transplants were considerably retarded over the longer periods of these experiments.

5. In some instances there was complete resorption of the transplants.

6. Under conditions of these experiments the period required for 1,2,5,6-dibenzanthracene to exert its influence on transplants of tooth germs was approximately the same as that for 20-methylcholanthrene.

REFERENCES

1. Barron, E. S. G.: Advances in Enzymology, Vol. 11, F. F. Nord, New York, 1951, Ed. Interscience Publications, Inc.
2. Barry, G., Cook, J. W., Haslewood, G. A. D., Hewett, C. L., Heiger, I., and Kennaway, E. L.: The Production of Cancer by Pure Hydrocarbons, Part III, *Proc. Roy. Soc. Lond., s. B.* **117**: 1935, 318-351.
3. Berenblum, I.: Cocarcinogenesis, *Brit. Med. Bull.* **4**: 343-347, 1946-47.
4. Berenblum, I., Holiday, E. R., and Jope, E. M.: Some Physical Methods of Investigating Carcinogenic Hydrocarbons, *Brit. Med. Bull.* **4**: 326-330, 1946-47.
5. Boyland, E.: Chemical Carcinogenesis and Experimental Chemotherapy of Cancer, *Yale J. Biol. & Med.* **20**: 321-341, 1947-48.
6. *Idem*: The Biochemistry of Abnormalities in Cell Division, *Biochim. et Biophys. Acta* **4**: 293-300, 1950.
7. Boyland, E., and Weigert, F.: Metabolism of Carcinogenic Compounds, *Brit. Med. Bull.* **4**: 354-359, 1946-47.
8. Bryan, W. R., and Shimkin, M. B.: Quantitative Analysis of Dose-Response Data Obtained With Three Carcinogenic Hydrocarbons in Strain C3H Male Mice, *J. Nat. Cancer Inst.* **3**: 503-531, 1942-43.
9. Crabtree, H. G.: Anti-Carcinogenesis, *Brit. Med. Bull.* **4**: 345-347, 1946-47.
10. Fleming, H. S.: Homologous and Heterologous Growth of Transplanted Tooth Germs, *J. D. Res.* **31**: 166-188, 1952.
11. *Idem*: Early Effect of Methylcholanthrene on Transplanted Tooth Germs: A Histopathogenic Study, *J. D. Res.* **31**: 398-411, 1952.
12. *Idem*: Effect of Methylcholanthrene on Transplanted Tooth Germs: Additional Studies, *J. D. Res.* **33**: 531-537, 1954.
13. Greene, H. S. N.: The Production of Carcinoma and Sarcoma in Transplantable Embryonic Tissues, *Science* **101**: 644-645, 1945.
14. Greenstein, J. P.: Biochemistry of Cancer; ed. 2, New York, 1954, Academic Press Inc.
15. Greenstein, J. P., and Haddow, A.: Advances in Cancer Research, Vol. I, New York, 1953, Academic Press Inc.
16. Haddow, A., and Kon, G. A. R.: Chemistry of Carcinogenic Compounds; *Brit. Med. Bull.* **4**: 314-325, 1946-47.
17. Haddow, A.: Mode of Action of Chemical Carcinogens, *Brit. Med. Bull.* **4**: 331-342, 1946-47.
18. Heidelberger, C., and Jones, H. B.: The Distribution of Radioactivity in Mice Following the Administration of 1,2,5,6-Dibenzanthracene Labelled in the 9- and 10-Positions With Carbon, *Cancer* **1**: 252-260, 1948.

19. Rigdon, R. H.: Tumors Produced by Methylcholanthrene in the Duck, *Arch. Path.* **54**: 368-377, 1952.
20. Rondoni, P., and Barbieri, G. P.: The Action of Some Carcinogenic Compounds on SH-activated Enzymes, *Enzymologia* XIV; 15, III: 10-14, 1950.
21. Rous, P., and Smith, W. E.: The Neoplastic Potentialities of Mouse Embryo Tissues. I. The Findings With the Skin of C strain Embryos Transplanted to Adult Animals, *J. Exper. Med.* **81**: 597-620, 1945.
22. Smith, W. E., and Rous, P.: The Neoplastic Potentialities of Mouse Embryo Tissues. II. Contributary Experiments; Experiments With the Skin of C3H and Webster-Swiss Embryos; General Considerations, *J. Exper. Med.* **81**: 621-646, 1945.
23. Tagashira, Y., Takeda, S., Kawano, K., and Amano, S.: Continuous pH Measuring by Means of Microglass Electrode Inserted in Living Normal and Tumor Tissues (2nd Report), With an Additional Report on Interaction of SH-Group of Animal Protein With Carcinogenic Agents in the Carcinogenetic Mechanism, *Gann* **45**: 99-100, 1954.
24. Thoma, K.: Oral Pathology, ed. 2, The C. V. Mosby Company, St. Louis, 1944.
25. Ward, G. E., and Hendrick, J. W.: Tumors of The Head and Neck, Baltimore, 1950, The Williams & Wilkins Company.
26. Weist, W. G., and Heidelberger, C.: The Interaction of Carcinogenic Hydrocarbons With Tissue Constituents. II. 1:2:5:6-Dibenzanthracene-9, 10-C^{14} in Skin, *Cancer Res.* **13**: 250-254, 1953.
27. Wood, J. L., and Kraynak, M. E.: A Decrease in Plasma Sulfhydryl Content in the Dog After Intravenous Administration of 3, 4-Benzpyrene, *Cancer Res.* **13**: 358-360, 1953.

NOTICE OF MEETING

I.A.D.R.—35th Annual Meeting
The Claridge Hotel, Atlantic City, N. J.
March 21, 22, 23, 1957

Dental Materials Group
March 22, 23, 24, 1957
Registration Fee $3.00

Preprinted abstracts will be supplied
before the meeting to those who register
by mail prior to February 15, 1957. Write to

Dr. Dan Y. Burrill
129 E. Broadway
Louisville 2, Ky.

stating name, address, affiliation, Section
of Group and enclosing registration fee of $3.00

SUPPORTING ASSOCIATES*

OF

THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

AND

THE JOURNAL OF DENTAL RESEARCH

Acralite Company, Inc., New York, N. Y.
Julius Aderer, Inc., New York, N. Y.
Baker & Company, Inc., Newark, N. J.
Bristol-Myers, New York, N. Y.
L. D. Caulk Company, Milford, Del.
Coe Laboratories, Inc., Chicago, Ill.
Colgate Palmolive Company, Jersey City, N. J.
Cook-Waite Laboratories, Inc., New York, N. Y.
Crescent Dental Manufacturing Company, Chicago, Ill.
Densco, Incorporated, Denver, Colo.
Dental Materials Group, I.A.D.R., Indianapolis, Ind.
Dentists' Supply Company of N. Y., York, Pa.
Hanau Engineering Company, Inc., Buffalo, N. Y.
International Nickel Company, New York, N. Y.
J. F. Jelenko & Company, Inc., New York, N. Y.
H. D. Justi and Sons, Inc., Philadelphia, Pa.
Luxene, Inc., New York, N. Y.
The J. M. Ney Company, Hartford, Conn.
Precious Metals Research Works, Inc., New York, N. Y.
Procter & Gamble Company, Cincinnati, Ohio
Ritter Company, Inc., Rochester, N. Y.
Ticonium Division, Consolidated Metal Products Corp., Albany, N. Y.
The S. S. White Dental Manufacturing Company, Philadelphia, Pa.
Williams Gold Refining Company, Inc., Buffalo, N. Y.

*Supporting Associates have made contributions to the Association for support of the JOURNAL. These contributions are in multiples of one hundred dollars.

CONTENTS FOR FEBRUARY, 1957 (*Cont'd*)

Acid Production From Different Carbohydrate Foods in Plaque and Saliva. T. G. Ludwig, B.D.S., M.S., and B. G. Bibby, B.D.S., Ph.D., D.M.D., Rochester, N. Y. 56

Further Observations Upon the Caries-Producing Potentialities of Various Foodstuffs. T. G. Ludwig, B.D.S., M.S., and B. G. Bibby, B.D.S., Ph.D., D.M.D., Rochester, N. Y. ---------- 61

The Effect of Alloxan Diabetes on Caries Incidence in the Albino Rat. Martin S. Nichols and James H. Shaw, Boston, Mass. ---------- 68

Inhibition of Acid Production of Salivary Sediment by Ethers, Esters, Phenols, and Alcohols. R. S. Manly, Ph.D., and Gladys Hargreaves, M.S., Boston, Mass. ---- 75

Several Properties of Saliva From Hunt-Hoppert Caries-Resistant and Caries-Susceptible Rats. S. Rosen, M. A. Benarde, F. W. Fabian, H. R. Hunt, and C. A. Hoppert, East Lansing, Mich. ---------- 87

Decreased Vascularity of the Dental Pulp. I. The Effects of Unilateral Ligation of Major Vessels to the Teeth and of Combined Ligation-Salivary Adenectomy on Dental Caries Experience in the Rat. Harold E. Brewer, Joseph C. Muhler, and Robert B. Fischer, Bloomington, Ind. ---------- 92

Experimental Dental Caries. IX. Relationship of Thyroid Function to Dental Caries Experience in Salivary-Adenectomized Rats. Joseph C. Muhler and William G. Shafer, Bloomington and Indianapolis, Ind. ---------- 95

Biochemical Analysis of Human Salivary Mucin. I. The Possibility of Cholesterol in Mucin. M. Irwin and A. G. Leaver, Dunedin, New Zealand ---------- 100

Egg Yolk Reactions by Organisms From the Oral Cavity of Man. Genevieve D. Roth, D.D.S., and Hugh I. Myers, Ph.D., Kansas City, Mo. ---------- 104

A Comparative Study of Human Serum and Salivary Antibody Titers in Cases of Brucella Melitensis Infections. M. G. Wheatcroft, Cairo, Egypt ---------- 112

Effects of Buffered Solutions of Sodium Fluoride and Stannous Fluoride on the Solubility of Powdered Enamel Using Repeated Decalcification. Robert H. Walsh, Ph.D., William H. Nebergall, Ph.D., Joseph C. Muhler, D.D.S., Ph.D., and Harry G. Day, Sc.D., Bloomington, Ind. ---------- 118

Relative Caries-Inhibiting Value of Topically Applied Sodium Silicofluoride and Sodium Fluoride. John K. Peterson, D.D.S., M.P.H., and Wm. A. Jordan, D.D.S., M.P.H., Minneapolis, Minn. ---------- 124

The Inhibitory Effect of Sodium N-Lauroyl Sarcosinate on Oral Lactobacilli When Grown on Various Media. I. L. Shklair, Ph.D., K. C. Hoerman, D.D.S., M.S., and H. R. Englander, D.D.S, M.P.H., Great Lakes, Ill. ---------- 129

A Clinical Study of Blood Pressure Responses to Epinephrine-Containing Local Anesthetic Solutions. Frank M. McCarthy, M.D., D.D.S., M.S., Los Angeles, Calif. 132

Histochemical and Electron Microscopy Studies of Dentin in Tryptophan-Deficient Rats. Sol Bernick and L. A. Bavetta, Los Angeles, Calif. ---------- 142

The Relation of Oxygen Pressure to Endogenous Oxygen Consumption in Bovine Dental Pulp. Alton K. Fisher, Erwin R. Schumacher, Norman P. Robinson, Delmar D. Albers, and Gary P. Sharbondy, Iowa City, Iowa ---------- 150

Effect of 1,2,5,6-Dibenzanthracene on Transplanted Tooth Germs. Harold S. Fleming, D.M.D., M.Sc., D.Sc., New Haven, Conn. ---------- 153

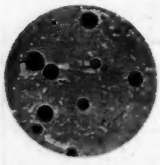
STRENGTH FROM STRUCTURE

Observe how a bird builds its nest—how it binds and weaves and cements—the instinctive use of structure to build strength.

Nature builds strong natural teeth with the same [illegible] care by developing a dense, homogenous internal structure . . . and man-made teeth must be fabricated from a material which has a similar internal structure in order to provide the maximum possible strength for the wearer of artificial dentures.

The vacuum fired porcelain of Trubyte Bioform Teeth approaches this natural perfection of structural material. The internal and external gases causing the voids and air bubbles in conventional porcelain have been substantially removed. A denser, more homogenous material results that allows grinding and polishing without risk of chipping or flaking.

CONVENTIONAL AIR FIRED PORCELAIN



TRUBYTE BIOFORM VACUUM FIRED PORCELAIN

Note in these two photomicrographs how the denser, more homogenous structure of vacuum fired porcelain is substantially free from the voids and bubbles found in conventional porcelain.

For greater strength and more beautiful esthetics for all your complete and partial denture requirements, specify the one and only Trubyte Bioform Vacuum Fired Porcelain Anteriors and Posteriors.

trubyte Bioform® teeth

The VACUUM FIRED PORCELAIN Teeth

THE DENTISTS' SUPPLY COMPANY OF N.Y.

YORK, PENNSYLVANIA